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Novel spectral manipulations for determinations of Tolnaftate along with related toxic compounds: Drug profiling and a comparative study

Raghda A. Emam *, Maha M. Abdelrahman, Eglal A. Abdelaleem, Nouruddin W. Ali

Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Beni-Suef University, Alshaheed Shehata Ahmed Hegazy st., 62514 Beni-Suef, Egypt

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ABSTRACT

A comparative study using novel quadruple divisor and mean centering of ratio spectra spectrophotometric methods was developed for resolution of five- component mixture of Tolnaftate, β-naphthol (Tolnaftate alkaline degradation product and its toxic impurity), methyl(m-tolyl)carbamic acid (Tolnaftate alkaline degradation product), *N*-methyl-m-toluidine (Tolnaftate toxic impurity) and methyl paraben (as a preservative). For the novel quadruple divisor method, each component in the quinary mixture was determined by dividing the quinary mixture spectrum by a sum of standard spectrum of equal concentration of the other four components as a quadruple divisor. First derivative of each ratio spectra was then obtained which allowed selective determination of each component without interference from other components in the mixture. The second method was mean centering of ratio spectra that depended on utilizing the mean centered ratio spectra in four successive steps leading to enhancement of the signal to noise ratio. The absorption spectra of the five studied components were recorded in the wavelength range of 210–350 nm. The mean centered fourth ratio spectra amplitudes for each component were used for its determination. The developed methods were successfully applied for determination of laboratory prepared quinary mixtures to ensure method's specificity, then, were further applied on Tinea Cure® cream where no interference from excipients. For the first time, Tolnaftate was determined along with its toxic impurity; β-naphthol, that could be absorbed by the skin, causing systemic toxic effects, unlike Tolnaftate that poorly absorbed, indicating the significance of this work. The proposed methods were statistically compared with each other and with the reference method. Furthermore, ICH guidelines were followed for their validation.

1. Introduction

Tolnaftate (TF) is chemically designated as *O*-naphthalen-2-yl-methyl(3-methylphenyl)thiocarbamate [1]. It is an antifungal drug that used topically in the treatment or prophylaxis of superficial dermatophyte infections and of pityriasis versicolor [2]. β-Naphthol (BN) is chemically designated as naphth-2-ol [2]. It was formerly used as an anthelmintic in hookworm and tapeworm infections, but it has been superseded by less toxic and more efficient drugs [2]. It has a potent parasiticidal effect and has been used topically in the treatment of scabies, ringworm, and other skin diseases [2]. BN is well absorbed by the skin and chronic dermal application or prolonged contact with BN may cause toxic effects such as skin sensitization, kidney impairment, hemolytic anemia, hemoglobinuria and retina opacities [3,4]. BN is referenced as TF impurity (A) and related substance [1]. Moreover, it is formerly reported as TF hydrolytic degradation product [5]. It is produced from alkaline hydrolysis of TF along with methyl(m-tolyl)carbamic acid (MTC) [5]. *N*,3-dimethylaniline (*N*-methyl-m-toluidine (NMT)), is referenced as TF impurity D [1]. It is TF related impurity arising during synthesis [6]. *N*-methyl-m-toluidine is toxic by different contact routes; (inhalation, oral and dermal) and prolonged or repeated exposure to it causes specific target organ toxicity [7]. Methyl paraben (MP) is a hydroxybenzoates preservative; alkyl esters of p-hydroxybenzoic acid, with antibacterial and antifungal properties. Methyl paraben is used as preservative in pharmaceutical preparations in usual concentrations of up to 0.25% [2].

A review in the literature revealed few methods for quantitative determination of TF including Pharmacopeial [1] and non-Pharmacopeial spectrophotometric methods [5,8–10]. Different HPLC methods were reported for determination of TF in bulk powder, different pharmaceutical formulations or human skin samples [11–13], and in combination with other drugs [14–16]. An isocratic supercritical fluid chromatographic method was also reported for determination of TF and its related impurities (NMT and β-naphthol-1-chlorothiocarbamate) [8].

Abbreviations: BN, β-Naphthol; MTC, Methyl(m-tolyl)carbamic acid; MP, Methyl paraben; NMT, *N*-Methyl-m-toluidine; TF, Tolnaftate.

* Corresponding author.
E-mail addresses: raghdaemam88@gmail.com, Raghda011141@pharm.bsu.edu.eg (R.A. Emam).

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Current ICH guidelines [17] recommend the determination of drug minor components as impurities and degradation products along with the active drug for developing and validating a stability indicating assay method (SIAM). Hydrolysis is one of the most common routes of degradation because water is used as a solvent for most drugs and excipients or due to moisture in the air [18]. Accordingly, TF was subjected to alkaline hydrolysis as mentioned in the literature [5], and the obtained degradation products (BN and MTC) were isolated, identified and characterized by different spectroscopic techniques (IR, MS spectroscopy and 1H NMR). Moreover, NMT was synthesized by methylation of m-toluidine with dimethylsulphate [19]. The purity of the methylated product was checked by TLC, IR, 1H NMR and mass spectrometry.

To the best of our knowledge, there is no reported method for determination of TF in presence of its impurity along with degradation products. This determination has to be done due to the reported toxicity of BN and NMT and the different pharmacological action of BN than the parent drug. Also, unlike TF, BN is well absorbed by the skin then causes systemic toxic effects that confirm the importance of this determination due to BN toxicity. The UV spectra of related components are usually expected to be overlapped. Mean centering of ratio spectra spectrophotometric methods for precise resolution of multi-component mixtures were reported [20, 21]. The novel quadruple divisor method could be considered as an extension of the recently reported triple divisor spectrophotometric method [20].

The work in this manuscript was planned to determine and resolve severely overlapped spectra of TF, toxic BN, MTC, toxic NMT and MP by mean centering of ratio spectra and novel quadruple divisor spectrophotometric methods.

The proposed spectrophotometric methods had the advantages of being cost effective and could be used for resolution of complex drug matrix without previous separation or extraction steps or need of complicated apparatus like chromatographic methods.

1.1. Theoretical background

1.1.1. Quadruple divisor spectrophotometric method

In the quadruple divisor spectrophotometric method, for determination of TF in a quinary mixture containing TF, BN, MTC, NMT and MP, the spectra of the mixtures were divided by the sum standard spectra of equal concentrations of BN, MTC, NMT and MP as (a quadruple divisor) as following:

$$\text{As}: A_m = \propto_{TF} C_{TF} + \propto_{BN} C_{BN} + \propto_{MTC} C_{MTC} + \propto_{NMT} C_{NMT} + \propto_{MP} C_{MP}$$

and

$$A_{qd} = \propto_{BN} C^0{}_{BN} + \propto_{MTC} C^0{}_{MTC} + \propto_{NMT} C^0{}_{NMT} + \propto_{MP} C^0{}_{M}$$

Where, Am is the absorbance vector of the mixture, Aqd, is the absorbance vector of the quadruple divisor, ∝TF, ∝BN, ∝MTC, ∝NMT and ∝MP are the absorptivity vectors, C_{TF}, C_{BN}, C_{MTC}, C_{NMT} and C_{MP} are the concentrations of TF, BN, MTC, NMT and MP in the mixture, respectively, and C^0_{BN}, C^0_{MTC}, C^0_{NMT} and C^0_{MP} are the standard concentration of BN, MTC, NMT and MP, respectively, in the quadruple divisor. The first derivative of the resulted ratio spectra was then obtained as following:

$$\frac{d}{d\lambda}\frac{Am}{\propto BN\ C0BN + \propto MTC\ C0MTC + \propto NMT\ C0NMT + \propto MP\ C0\ MP} = \frac{d}{d\lambda}\frac{\propto TF\ CTF + \propto BN\ CBN + \propto MTC\ CMTC + \propto NMT\ CNMT + \propto MP\ CMP}{\propto BN\ C0BN + \propto MTC\ C0MTC + \propto NMT\ C0NMT + \propto MP\ C0\ MP} =$$

$$= \frac{d}{d\lambda}\left[\frac{\propto TF\ CTF}{\propto BN\ C0BN + \propto MTC\ C0MTC + \propto NMT\ C0NMT + \propto MP\ C0MP} + Constant\right]$$

$$= \frac{d}{d\lambda}\left[\frac{\propto TF\ CTF}{\propto BN\ C0BN + \propto MTC\ C0MTC + \propto NMT\ C0NMT + \propto MP\ C0MP}\right] + Zero$$

The first derivative amplitudes measured in the last equation are only dependent on concentration values, C_{TF}, C^0_{TF}, C^0_{BN}, C^0_{MTC}, C^0_{NMT} and C^0_{MP} (C^0 is standard concentration), but are independent on the concentration values C_{BN}, C_{MTC}, C_{NMT} and C_{MP} in the quinary mixture.

A calibration curve could be constructed by plotting the first derivative of the obtained ratio spectrum amplitude at any wavelength; $\frac{d}{d\lambda}[(Am/\propto BN\ C0BN + \propto MTC\ C0MTC + \propto NMT\ C0NMT + \propto MP\ C0\ MP]$, versus concentration of TF and a calculated regression equation was obtained from which TF concentration in the quinary mixture could be determined. The concentrations of the other components, BN, MTC, NMT and MP, can be determined individually by similar procedures to that of TF.

1.1.2. Mean centering of ratio spectra spectrophotometric method

Issa et al. [21] provided the MCR theoretical background used for resolving quinary mixtures as follow, if a mixture of five compounds, TF, BN, MTC, NMT and MP obeyed Beer's law over the whole specified wavelength range, then

$$A_m = \propto_{TF} C_{TF} + \propto_{BN} C_{BN} + \propto_{MTC} C_{MTC} + \propto_{NMT} C_{NMT} + \propto_{MP} C_{MP} \quad (1)$$

where A_m is the absorbance vector of the mixture, $\propto_{TF}$, $\propto_{BN}$, $\propto_{MTC}$, $\propto_{NMT}$ and $\propto_{MP}$ are the absorptivity vectors, C_{TF}, C_{BN}, C_{MTC}, C_{NMT} and C_{MP} are the concentrations of TF, BN, MTC, NMT and MP in the mixture, respectively.

For determination of TF, Eq. (1) is divided by $\propto_{BN}$ (the first divisor, D1), corresponding to standard solution spectrum of BN, so the first ratio spectrum is gained.

$$\frac{Am}{\propto BN} = \frac{\propto TF\ CTF}{\propto BN} + CBN + \frac{\propto MTC\ CMTC}{\propto BN} + \frac{\propto NMT\ CNMT}{\propto BN} + \frac{\propto MP\ CMP}{\propto BN} \quad (2)$$

If Eq. (2) is mean centered, then

$$mc\frac{Am}{D1} = mc\frac{\propto TF\ CTF}{D1} + mc\frac{\propto MTC\ CMTC}{D1} + mc\frac{\propto NMT\ CNMT}{D1} + mc\frac{\propto MP\ CMP}{D1} \quad (3)$$

If Eq. (3) is divided by $mc\frac{\propto MTC}{\propto BN} = mc\frac{\propto MTC}{D1} =$ the second divisor, D2, so the second ratio spectrum is obtained

$$\frac{mc\frac{Am}{D1}}{D2} = \frac{mc\frac{\propto TF\ CTF}{D1}}{D2} + CMTC + \frac{mc\frac{\propto NMT\ CNMT}{D1}}{D2} + \frac{mc\frac{\propto MP\ CMP}{D1}}{D2} \quad (4)$$

If Eq. (4) is mean centered, then

$$mc\frac{mc\frac{Am}{D1}}{D2} = mc\frac{mc\frac{\propto TF\ CTF}{D1}}{D2} + mc\frac{mc\frac{\propto NMT\ CNMT}{D1}}{D2} + mc\frac{mc\frac{\propto MP\ CMP}{D1}}{D2} \quad (5)$$

And if Eq. (5) is divided by $mc(\frac{mc\frac{\propto NMT}{D1}}{mc\frac{\propto MTC}{D1}}) = mc(\frac{mc\frac{\propto NMT}{D1}}{D2})$ (the third divisor, D3), this will results in the third ratio spectrum,

$$mc\frac{mc\frac{Am}{D1}}{D2}/D3 = mc\frac{mc\frac{\propto TF\ CTF}{D1}}{D2}/D3 + CNMT + mc\frac{mc\frac{\propto MP\ CMP}{D1}}{D2}/D3 \quad (6)$$

If Eq. (6) is mean centered, so,

$$mc\left(mc\frac{Am}{\frac{D1}{D2}}/D3\right) = mc\left(mc\frac{\propto TF\ CTF}{\frac{D1}{D2}}/D3\right) + mc\left(mc\frac{\propto MP\ CMP}{\frac{D1}{D2}}/D3\right) \quad (7)$$

and, if Eq. (7) is divided by $mc(\frac{mc\frac{D1}{D2}}{D2}/D3)$, (the fourth divisor, D4), this will result in the fourth ratio spectrum:

$$mc\left(mc\frac{Am}{\frac{D1}{D2}}/D3\right)/D4 = mc\left(mc\frac{\propto TF\ CTF}{\frac{D1}{D2}}/D3\right)/D4 + CMP \quad (8)$$

If Eq. (8) is mean centered, so,

$$X = Z\,C_{TF} \quad (9)$$

where

$$X = mc\left(mc\left(mc\frac{Am}{\frac{D1}{D2}}/D3\right)/D4\right) \text{ and } Z = mc\left(mc\left(mc\frac{\propto TF\ CTF}{\frac{D1}{D2}}/D3\right)/D4\right)$$

The mean centered fourth ratio spectrum signal obtained in Eq. (9) is practically dependent only on C_{TF}, but is independent on C_{BN}, C_{MTC}, C_{NMT} and C_{MP} concentration values in the mixture. The concentrations of the other components (BN, MTC, NMT and MP) can be individually determined by similar procedures to that of TF.

2. Experimental

2.1. Instruments

A double beam UV–visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to IBM compatible computer. The software used was UVPC personal spectroscopy software V. 3.7.

Data concerning MCR was performed using PLS-Toolbox 2.0 running under MATLAB®, version (R2007b).

2.2. Materials and reagents

2.2.1. Pure standards

Tolnaftate (TF) and Methylparaben (MP) pure standards were kindly supplied by Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt as a gift and were used as working standards. Their purity was found to be 99.49% and 99.22%, respectively, according to the manufacturer certificate and 99.97% for TF, according to the reference spectrophotometric method.

2.2.2. Pharmaceutical formulation

Tinea Cure® Cream (B.N. 1670291) manufactured by Kahira Pharm. & Chem. Ind. Co. Cairo, Egypt, labeled to contain 1 g TF per 100 g cream.

2.2.3. Chemicals and solvents

All chemicals used throughout this work were of analytical grade and were used without further purification;

Table 1
The first, second, third and fourth divisors for TF, BN, MTC, NMT and MP in mean center of ratio spectra method.

	First divisor (D1)	Second divisor (D2)	Third divisor (D3)	Fourth divisor (D4)
TF	BN, 10 μg mL^{-1}	$mc\frac{\propto MTC}{\propto BN}$, using 10 μg mL^{-1} MTC	$mc(\frac{mc\frac{\propto NMT}{D1}}{D2})$, using 4 μg mL^{-1} NMT	$mc(\frac{mc\frac{\propto MP}{D1}}{D3})$, using 4 μg mL^{-1} MP
BN	TF, 6 μg mL^{-1}	$mc\frac{\propto MTC}{\propto TF}$, using 4 μg mL^{-1} MTC	$mc(\frac{mc\frac{\propto NMT}{D1}}{D2})$, using 4 μg mL^{-1} NMT	$mc(\frac{mc\frac{\propto MP}{D1}}{D3})$, using 4 μg mL^{-1} MP
MTC	TF, 10 μg mL^{-1}	$mc\frac{\propto BN}{\propto TF}$, using 10 μg mL^{-1} BN	$mc(\frac{mc\frac{\propto NMT}{D1}}{D2})$ using 10 μg mL^{-1} NMT	$mc(\frac{mc\frac{\propto MP}{D1}}{D3})$, using 10 μg mL^{-1} MP
NMT	TF, 8 μg mL^{-1}	$mc\frac{\propto BN}{\propto TF}$, using 4 μg mL^{-1} BN	$mc(\frac{mc\frac{\propto MTC}{D1}}{D2})$, using 4 μg mL^{-1} MTC	$mc(\frac{mc\frac{\propto MP}{D1}}{D3})$, using 4 μg mL^{-1} MP
MP	TF, 8 μg mL^{-1}	$mc\frac{\propto BN}{\propto TF}$, using 6 μg mL^{-1} BN	$mc(\frac{mc\frac{\propto MTC}{D1}}{D2})$, using 6 μg mL^{-1} MTC	$mc(\frac{mc\frac{\propto NMT}{D1}}{D3})$,using 6 μg mL^{-1} NMT

- Methanol, methylene chloride, toluene, potassium carbonate, sodium carbonate, m-toluidine, sodium hydroxide, hydrochloric acid (El-Nasr Pharmaceutical Chemicals Co., Abu Zabaal, Cairo, Egypt).
- Dimethyl sulphate, Methanol HPLC grade: (CHROMASOLV®, Sigma-Aldrich, Germany).

2.2.4. Preparation of degradation products

In the literature [5], TF was treated with 2 N NaOH solution in a boiling water bath for about 20 min to produce a fluorescent solution due to sodium β-naphtholate that was confirmed by UV and TLC in comparison with pure sodium β-naphtholate. But, there was no confirmation or separation of the second assumed degradation product mentioned in their work. Complete hydrolysis was done by heating TF solution in a water bath at 90–100 °C with 5 N NaOH solution in a volume of 1 mL for 25 min [5]. In our work, 0.5 g of pure powdered TF was dissolved in methanol and refluxed with 75 mL of 2 N NaOH solution at 80 °C. A fluorescent solution was produced after 30 min with smelling of H_2S gas odour and the degradation process was monitored by TLC using methylene chloride: toluene (5.5:4.5, by volume) as a developing system. Two new spots other than the drug spot were observed on TLC plate and the degradation process was continued for 4 h till complete degradation of TF. For separation of the two degradates from each other, the alkaline degradation solution was neutralized by dropping 2 N HCl solution till pH 6.8 at which degradates were precipitated, then isolated by filtration and allowed to dry. 20 mL of 1 N Na_2CO_3 solution was added to the powdered mixture of the two degradates and stirred for 1 h. on the magnetic stirrer at room temperature then the solution was filtered. The precipitate was washed with distilled water and left to dry (a), then the filtrate was dropped by 1 N HCl until precipitate was formed which isolated and allowed to dry (b). both (a) and (b) degradation products were subjected to IR, 1H NMR and MS spectroscopy to confirm their chemical structures and the spectral data confirmed that, degradate (a) was β-naphthol and degradate (b) was methyl(m-tolyl) carbamic acid, the same as the reported scheme of degradation [5].

2.2.5. Synthesis of NMT impurity

As mentioned in the literature [19], N-alkylated aromatic amines could be synthesized from the corresponding aromatic amines using an alkylating agent like alkyl sulphate or alkyl halide. In a round bottomed flask, a mixture of 0.01 M m-toluidine, 50 mL acetone (as a solvent) and 0.02 M anhydrous powdered K_2CO_3 (as a base and dehydrating agent) was stirred for 1 h at 30–35 °C then 0.011 M of dimethyl sulphate (DMS) (as alkylating agent) was added drop wise. The progress of the reaction was followed up by TLC using methanol: methylene chloride (4: 6, by volume) as a developing system and stirring was continued for 4 h. till disappearance of m-toluidine spot on TLC. The reaction mixture was filtered then the filtrate was dried at room temperature to evaporate the solvent. The solid residue was then collected and subjected to IR, 1H NMR and MS spectroscopy to confirm the formation of NMT.

2.2.6. Solutions

- Stock solutions of TF, BN, MTC, NMT and MP (1 mg mL^{-1}): 100 mg of each TF, BN, MTC, NMT and MP were accurately and separately weighed into 100-mL measuring flasks and the volume was then completed to the mark with methanol as a solvent.
- Working solutions of TF, BN, MTC, NMT and MP (0.1 mg mL^{-1}): They were prepared by diluting 10 mL from their respective stock solutions, into five separate 100-mL volumetric flasks and the volume was completed using methanol as a solvent.
- Laboratory prepared mixtures of TF, BN, MTC, NMT and MP: They were prepared from their respective working standard solutions (0.1 mg mL^{-1}) in different ratios using methanol as a solvent.
- Pharmaceutical formulation standard solution: An accurately weighed quantity of Tinea Cure® Cream equivalent to 100 mg TF was transferred into a 100-mL volumetric flask containing 75 mL methanol. The flask was then heated on a water bath until the cream melted then the mixture was sonicated for 15 min and the volume was then completed to the mark with methanol to obtain 1000 μg mL^{-1} TF pharmaceutical formulation stock solution from which a working solution of 100 μg mL^{-1} TF was quantitatively prepared using methanol as a solvent.

3. Procedure

3.1. Spectral characteristics

The absorption spectra of 10 μg mL^{-1} of each TF, BN, MTC, NMT and MP, were recorded over the range 200–400 nm using methanol as a blank.

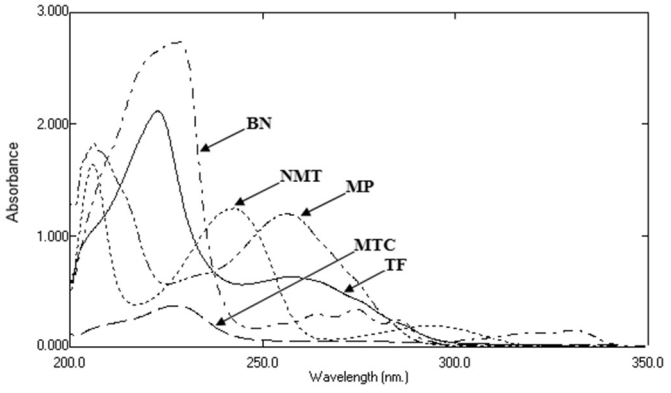

Fig. 1. Zero-order absorption spectra of 10 μg mL^{-1} each of Tolnaftate (TF), β-naphthol BN), Methyl-tolylcarbamic acid (MTC), *N*-methyl-m-toluidine (NMT) and Methyl paraben (MP) using methanol as a blank.

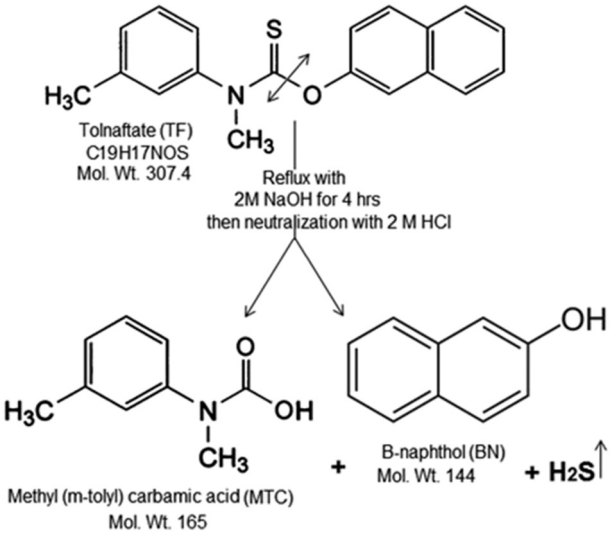

Fig. 2. Degradation pathway of Tolnaftate.

3.2. Linearity and construction of calibration curves

3.2.1. Quadruple divisor spectrophotometric method (QD)

Accurately measured aliquots equivalent to 10–120, 5–150, 20–280, 5–150 and 5–120 μg of TF, BN, MTC, NMT and MP, respectively, were transferred from their corresponding working standard solution (0.1 mg mL^{-1}) into five separate sets of 10-mL volumetric flasks and the volume was completed to the mark with methanol. Zero order absorption spectra of each set were recorded in the range of 215–350 nm.

For determination of TF, its absorption spectra were divided by sum standard spectra of equal concentrations of BN, MTC, NMT and MP (6 μg mL^{-1} of each) as a quadruple divisor. The first derivative (1D) of the resulted ratio spectra of TF was then obtained using $\Delta\lambda = 8$ and scaling factor = 1000, the amplitude values measured at 256 nm were plotted against the corresponding TF concentrations for constructing its calibration curve and the regression equation was calculated.

Similarly, BN, MTC, NMT and MP were determined following the abovementioned procedure for TF determination using a quadruple divisor of a mixture containing equal concentrations of the other four components (8, 4, 8 and 6 μg mL^{-1} of each, for determination of BN, MTC, NMT and MP, respectively) and the ^{1}D ratio spectra were then obtained using $\Delta\lambda$ and scaling factor, 8 and 1000, for MTC and MP determinations and 4 and 100 for BN and NMT determinations. The ^{1}D ratio spectra amplitudes were measured at 336.6, 288.4, 292 and 254 nm for BN, MTC, NMT and MP, respectively. Calibration curves were constructed between the amplitude values at the aforementioned wavelengths against the corresponding concentrations of each component and regression equations were then computed.

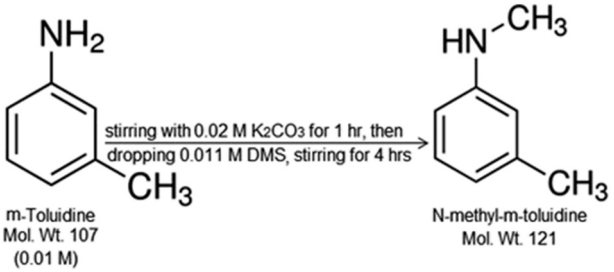

Fig. 3. Synthesis scheme of *N*-methyl-m-toluidine from m-toluidine.

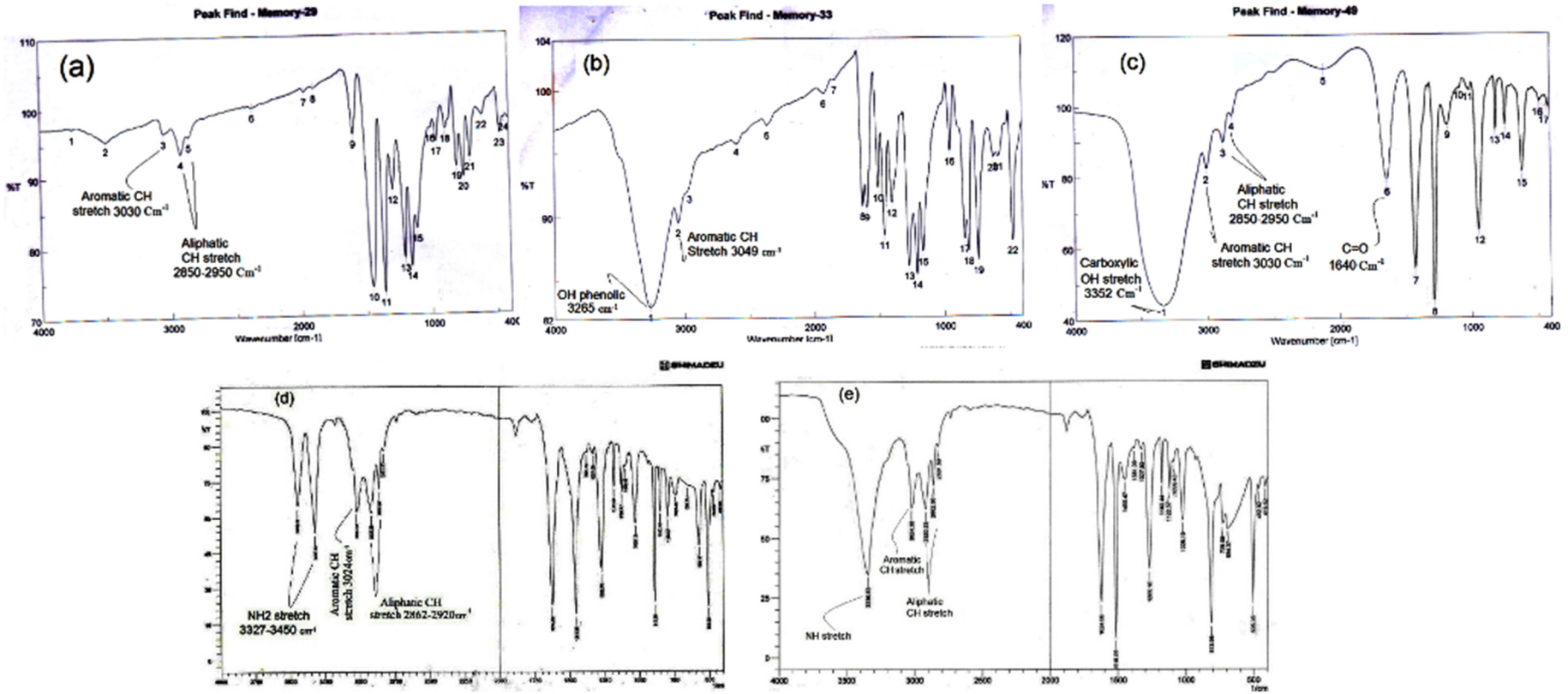

Fig. 4. IR spectra of (a): Tolnaftate, (b): β-naphthol, (c): Methyl-tolylcarbamic acid, (d): m-toluidine and (e): *N*-methyl-m-toluidine.

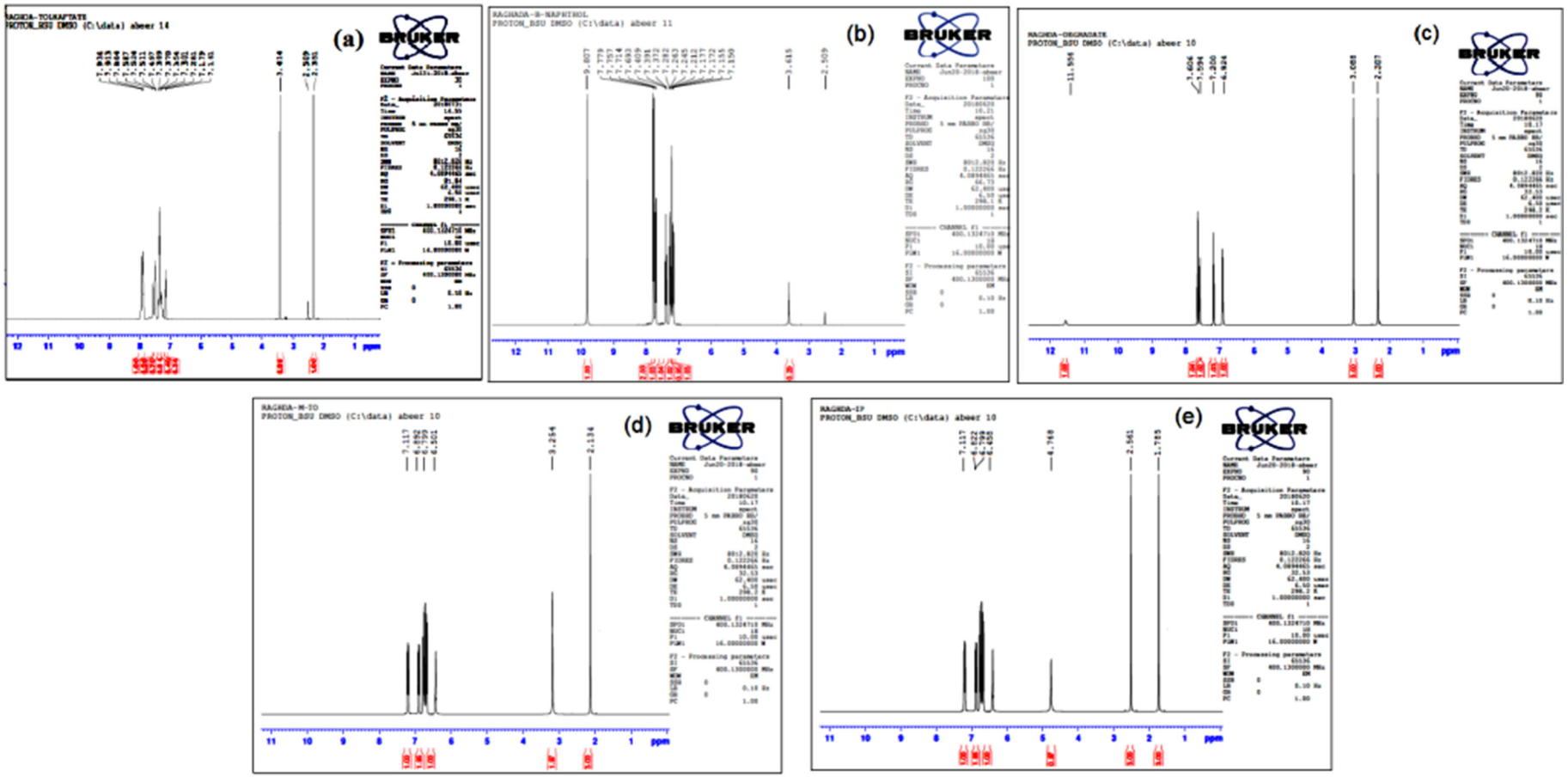

Fig. 5. 1H NMR spectra of (a): Tolnaftate, (b): *β*-naphthol, (c): Methyl-tolylcarbamic acid, (d): m-toluidine and (e): *N*-methyl-m-toluidine.

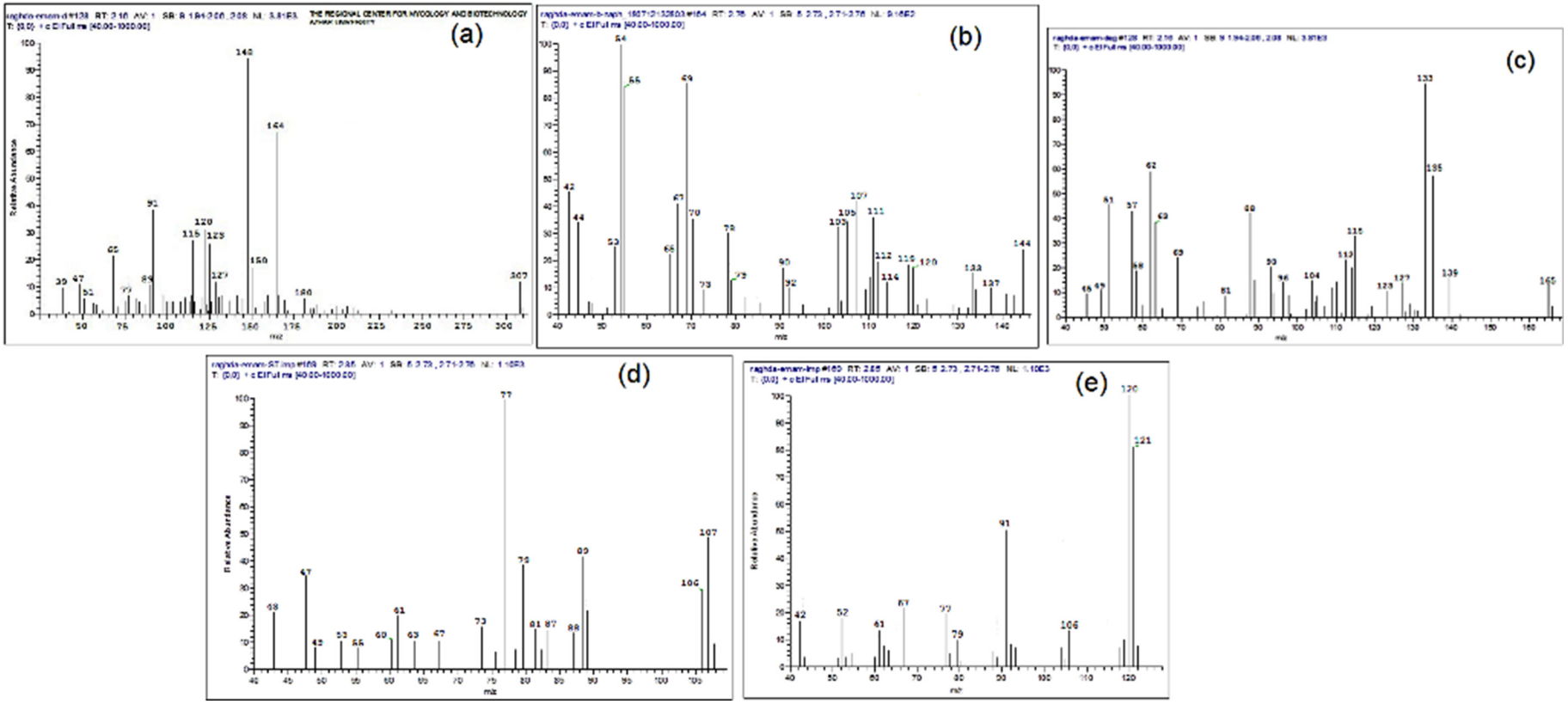

Fig. 6. Mass spectra of (a): Tolnaftate, (b): β-naphthol, (c): Methyl-tolylcarbamic acid, (d): m-toluidine and (e): *N*-methyl-m-toluidine.

3.2.2. Mean centering of ratio spectra spectrophotometric method (MCR)

Different aliquots equivalent to 10–120, 5–150, 20–280, 5–150 and 5–100 μg of TF, BN, MTC, NMT and MP, respectively, were accurately transferred from their corresponding working standard solutions (0.1 mg mL^{-1}) into five separate series of 10-mL volumetric flasks, the volumes were then completed with methanol. Zero order absorption spectra of each set were recorded over the range of 215–350 nm. For TF determination, the recorded spectra were divided by a standard spectrum of 10 μg mL^{-1} of BN, first divisor (D1), the obtained first ratio spectra were then mean centered. These vectors were then divided by $mc\frac{\propto MTC}{\propto BN}$, second divisor (D2), and the second ratio spectra were then mean centered. By the same procedure, using $mc\frac{\propto NMT}{\frac{\propto BN}{D2}}$ as a third divisor (D3), the third ratio spectra could be obtained then mean centered. Finally, the fourth ratio spectra were obtained by the same way, using $mc(\frac{mc\frac{MP}{D1}}{D2}/D3)$ as a fourth divisor (D4). For BN, MTC, NMT and MP determination, their respective recorded absorption spectra were manipulated similarly as with TF determination using their corresponding divisors concentrations mentioned in Table 1. The amplitudes of the mean centered fourth ratio spectra at 285 and 279 nm for TF and NMT, respectively, and at peak to peak 251–252, 310–311 and 312–313 nm for BN, MTC and MP, respectively, were measured and plotted against the corresponding concentrations of each component. Calibration curves were obtained and regression equations were computed.

3.3. Application to pharmaceutical formulation

Proper dilution for the pharmaceutical formulation standard stock solution (1000 μg mL^{-1}) was performed to obtain concentration within the linearity range of TF. The procedure illustrated under construction of calibration curve for each method was then followed and concentration of TF was determined using the computed regression equations.

3.4. Application to laboratory prepared mixtures

Different mixtures of TF, BN, MTC, NMT and MP in several ratios in concentrations within their linearity ranges were laboratory prepared from their respective working solutions (0.1 mg mL^{-1}). The UV spectra of prepared mixtures were recorded in the wavelength range of 215–350 nm. The procedures explained under linearity for each method were followed for calculation of the concentration of each component in the mixture using their corresponding regression equation.

4. Results and discussion

Although chromatographic techniques are widely used methods of analysis due to their high specificity and sensitivity [22], they are expensive, time consuming and need tedious preliminary separation steps. Spectrophotometric methods are simple and cost effective comparing to chromatographic methods, especially in laboratories lacking expensive chromatographic tools. Tolnaftate was subjected to alkaline hydrolysis producing BN and MTC. Both NMT and BN are TF toxic impurities and BN has different pharmacological action than parent drug (TF), Therefore, from the analytical point of view, it was important to develop a highly specific stability indicating spectrophotometric methods for determination of TF along with its toxic impurities (BN and NMT) and alkaline induced degradation product (MTC) without interference from MP present as a preservative in the pharmaceutical formulation. The work in this manuscript also aimed to provide an insight into alkaline induced degradation pathway and degradation products of TF and to elucidate the chemical structure of the degradation products and the synthesized impurity. The developed methods are considered as a profile for TF related components including toxic ones.

The UV-absorption spectra of TF and its related components along with MP, Fig. 1, showed severe overlap in the zero order that hindered their direct determination. Thus, from the analytical point of view, development of new spectrophotometric methods for resolution of such overlapped spectra without prior separation steps or using hyphenated instruments such as HPLC, was a must.

4.1. Structure elucidation of TF degradation products and synthesized impurity

Tolnaftate was subjected to alkaline hydrolysis under stress degradation conditions giving two degradation products (BN and MTC) due to cleavage of the thionoester bond in TF structure. Degradation pathway of TF was illustrated in Fig. 2. NMT was synthesized by methylation of m-toluidine using DMS as methylating agent in presence of

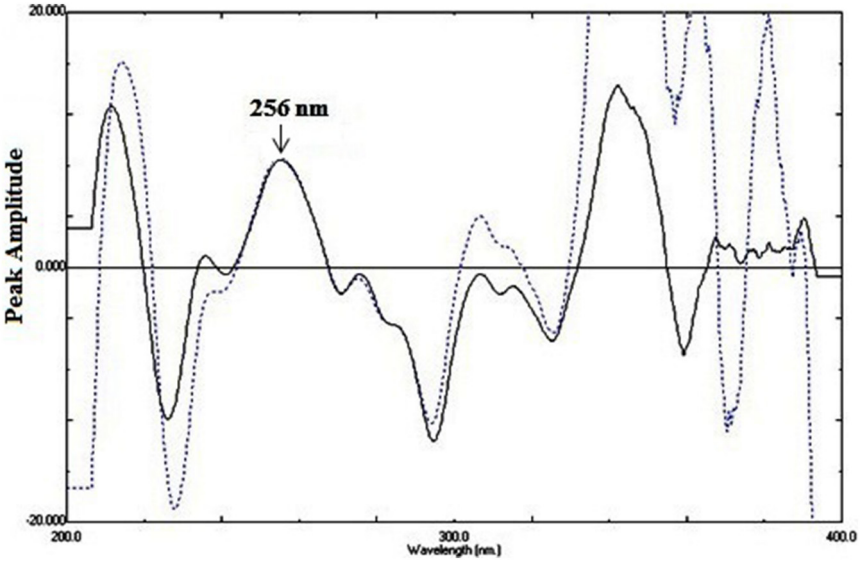

Fig. 7. The coincident spectra of the first derivative of the ratio spectra of 4 μg mL^{-1} pure TF (—) and a quinary mixture of TF, BN, MTC, NMT, and MP (4 μg mL^{-1} of each) (.....) using a mixture of 6 μg mL^{-1} each of BN, MTC, NMT and MP as a quadruple divisor and methanol as a blank.

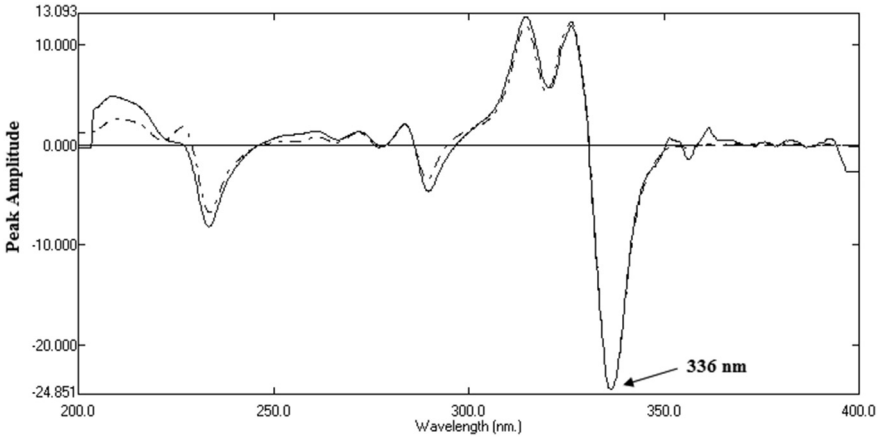

Fig. 8. The coincident spectra of the first derivative of the ratio spectra of 5 μg mL^{-1} BN (⁻·⁻·⁻) and a quinary mixture of TF, BN, MTC, NMT, and MP (10, 5, 5, 5 and 5 μg mL^{-1}, respectively) (__) using a mixture of 8 μg mL^{-1} each of TF, MTC, NMT and MP as a quadruple divisor and methanol as a blank.

anhydrous K_2CO_3 as a base and dehydrating agent. The scheme of synthesis was shown in Fig. 3. The degradation products and synthesized impurity were isolated as mentioned before and then characterized by IR, 1H NMR and MS analyses; Figs. 4-6.

4.1.1. IR spectral changes

Cleavage of the thionoester bond in TF structure by hydrolysis led to the formation of the corresponding phenol (BN) (Deg. I) and carboxylic acid (carbamic acid in MTC, Deg. II) accompanying by liberation of H_2S gas. The IR spectrum of TF, Fig. 4(a), showed aliphatic CH bands at 2850–2930 cm^{-1} and aromatic CH stretch at 3040 cm^{-1}. The IR spectrum of Deg. I, Fig. 4(b), showed disappearance of the aliphatic CH bands but not the aromatic one and appearance of new band at 3265 cm^{-1} indicating the formation of new phenolic OH group of BN. Presence of the same CH bands of TF and appearance of OH broad band at 3352 cm^{-1} and a characteristic amide C═O stretch at 1640 cm^{-1} in the IR spectra of Deg. II, Fig. 4(C), confirmed the presence of carbamic acid group (—N—COOH) and formation of MTC. Meta-toluidine IR spectrum, Fig. 4(d), showed a forked peak at 3327–3450 cm^{-1} characteristic to NH_2 group that disappeared in the IR spectrum of the synthesized product and a new sharp single peak at 3336 cm^{-1} was appeared instead, as shown in Fig. 4(e), indicating *N*-methylation of m-toluidine and conversion of the primary amine to a secondary amine to form *N*-methyl-m-toluidine.

4.1.2. 1H NMR spectral changes

Tolnaftate 1H NMR spectrum showed two singlet signals at δ 2.351 and 3.414 that were assigned to the N-substituted methyl group protons and meta-substituted methyl group protons on benzene ring. These signals were disappeared in the 1H NMR spectrum of Deg. I with the appearance of phenolic proton chemical shift at 9.807 ppm that confirmed the formation of BN, as shown in Fig. 5(a & b). In the 1H NMR spectrum of Deg. II, the methyl groups protons were found to resonate at δ 2.307 and 3.088 and a new very broad singlet signal was appeared at δ 11.556 due to carboxylic group proton that confirmed the formation of MTC as a degradation product as shown in Fig. 5(c). The 1H NMR spectrum of m-toluidine, Fig. 5(d), showed singlet signal at δ 2.262 due to meta substituted methyl group protons on the benzene ring and a singlet signal at δ 3.254 due to two protons of the primary amine (NH_2) (integration 2 protons). In the H^1NMR spectrum of the synthesized impurity, Fig. 5 (e), another singlet signal at δ 1.785 was appeared due to the new methyl group protons added by methylation and conversion of the primary amine to a secondary one that was confirmed by the appearance of a singlet signal at δ 4.768 due to only one proton of NH (integration 1 proton) as shown in Fig. 5(e).

4.1.3. Mass spectra

The electron impact showed mass ion peak at *m/z* 307 corresponding to the intact drug, Fig. 6(a). The electron impact of Deg. I showed

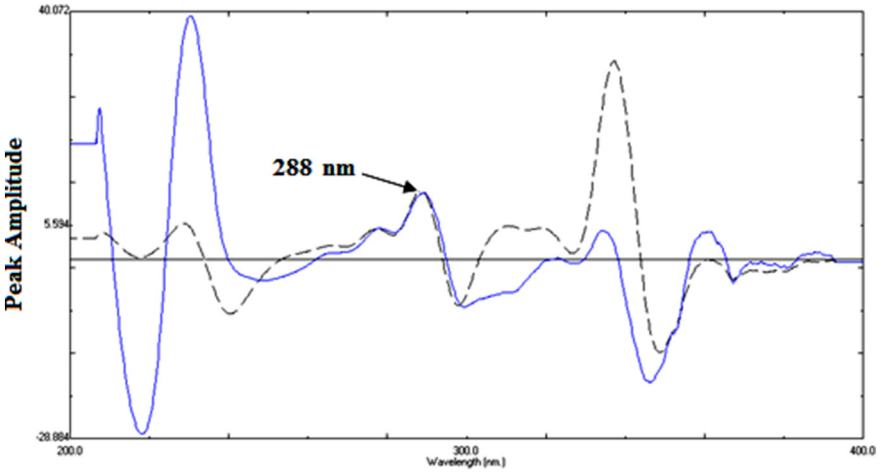

Fig. 9. The coincident spectra of the first derivative of the ratio spectra of 10 μg mL^{-1} MTC (— — -) and a quinary mixture of TF, BN, MTC, NMT, and MP (5, 5, 10, 5 and 5 μg mL^{-1}, respectively) (__) using a mixture of 4 μg mL^{-1} each of TF, MTC, NMT and MP as a quadruple divisor and methanol as a blank.

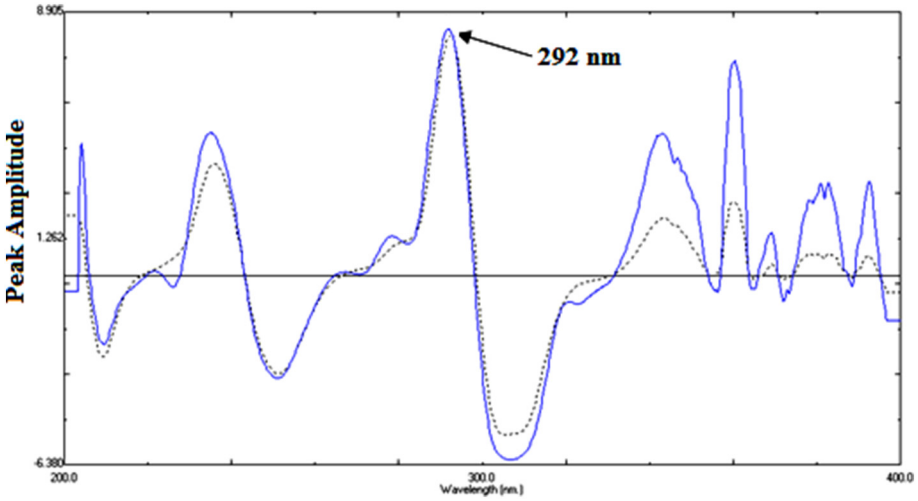

Fig. 10. The coincident spectra of the first derivative of the ratio spectra of 5 μg mL^{-1} NMT (….) and a quinary mixture of TF, BN, MTC, NMT, and MP (10, 5, 5, 5 and 5 μg mL^{-1}, respectively) (__) using a mixture of 8 μg mL^{-1} each of TF, MTC, NMT and MP as a quadruple divisor and methanol as a blank.

mass ion peak at 144 m/z corresponding to the molecular weight of the expected degradate (BN), while that of Deg. II showed mass ion peak of 165 *m/z* corresponding to the molecular weight of the expected degradate (MTC) and confirmed its identity and IR and H^1NMR interpretations. Moreover, the synthesized impurity mass spectrum showed parent peak at *m/z* 121 corresponding to NMT molecular weight and confirming that, methylation had been done and affirming the IR and H^1NMR interpretations. Mass spectra are shown in Fig. 6(a)(e).

4.2. Methods development and optimization

4.2.1. Quadruple divisor spectrophotometric method (QD)

For development of QD method, different concentrations of the quadruple divisor were tried out. Sum standard spectra of 6 μg mL^{-1} each of BN, MTC, NMT and MP were chosen as the optimum quadruple divisor for specific determination of TF. For BN, MTC, NMT and MP determinations, 8, 4, 8 and 6 μg mL^{-1} of the other four components were used as quadruple divisors, respectively. Another key element in the QD method development was wavelength selection. Different wavelengths were tried to select the optimum one, at which, the first derivative signal maximum or trough amplitude of the pure component, to be analyzed, synchronized with the first derivative amplitude of its quinary mixture to get rid of the interference of the other four components in the quinary mixture, as shown in Fig. 7-11. For determination of TF, the first derivative amplitude of the quinary mixture measured at wavelength maximum of 256 nm were coincided with that obtained from pure TF of the same concentration as in the quinary mixture, ensuring elimination of the interference from the other four components. Similarly, BN, MTC, NMT and MP were measured at 336.6, 288.4, 292 and 254 nm, respectively; corresponding to a maximum or minimum wavelength of the first derivative of the ratio spectra obtained using a mixture of the other four components as a divisor, Fig. 7-11.

4.2.2. Mean centering of ratio spectra spectrophotometric method (MCR)

For MCR method optimization, selection of a suitable divisor concentration was the key element in MCR method optimization for resolving the highly overlapped spectra under study. Different divisors concentrations were tested for each component determination in the quinary mixture. The optimum divisors concentrations used, that gave optimum specificity and results reproducibility, were listed in Table 1. As illustrated under linearity, for determination of TF, BN, MTC, NMT and MP concentrations, mean centered fourth ratio spectra were obtained and their amplitudes at 285 nm, Fig. 12(a), at peak to peak 251–252 nm, Fig. 12(b), 310–311 nm, Fig. 12(c), 279 nm, Fig. 12(d) and at peak to peak 312–313 nm, Fig. 12(e), respectively, were measured and plotted against their corresponding concentrations.

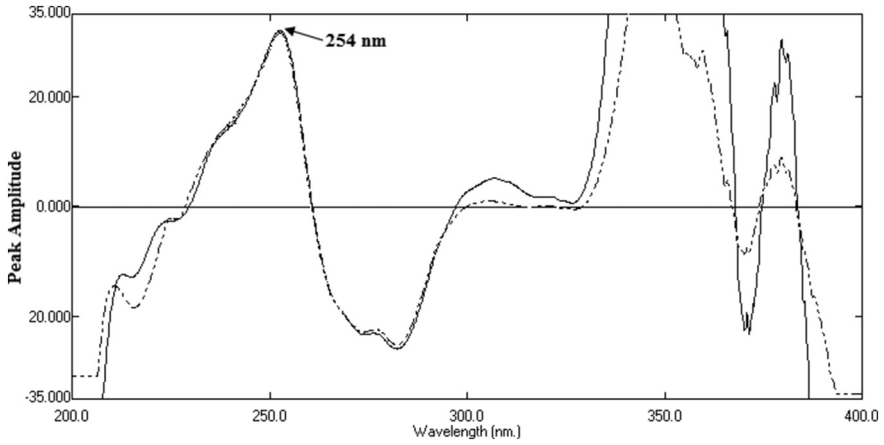

Fig. 11. The coincident spectra of the first derivative of the ratio spectra of 4 μg mL^{-1} MP (-..-..-) and a quinary mixture of TF, BN, MTC, NMT, and MP (4 μg mL^{-1} of each) (__) using a mixture of 6 μg mL^{-1} each of TF, MTC, NMT and MP as a quadruple divisor and methanol as a blank.

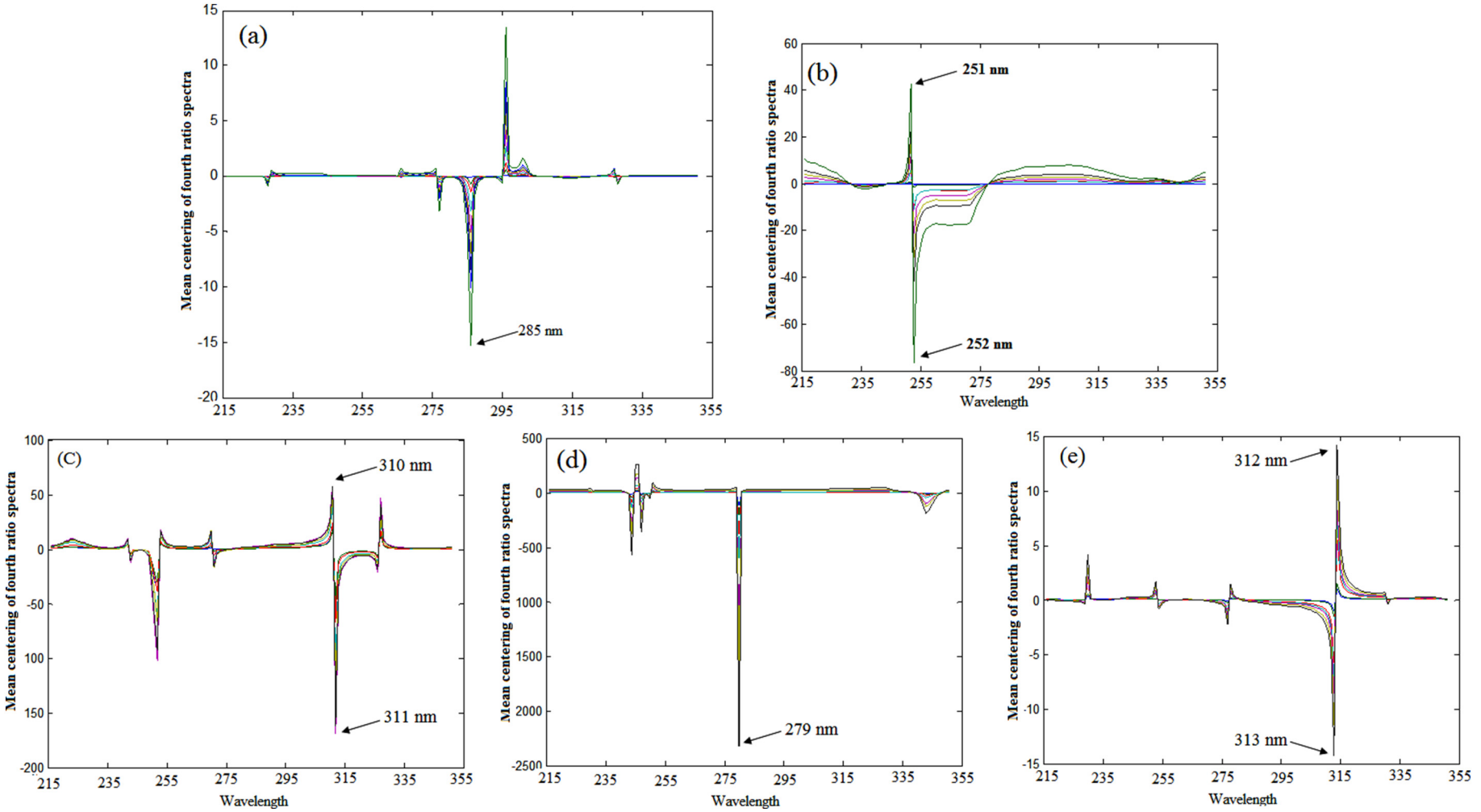

Fig. 12. The mean centered ratio spectra of (a): Tolnaftate, (b): *β*-naphthol, (c): Methyl-tolylcarbamic acid (d) *N*-methyl-m-toluidine and (e): Methyl paraben using methanol as a blank.

Table 2
Regression parameters of the proposed QD and MCR spectrophotometric methods for determination of TF, BN, MTC, NMT and MP.

Parameters	QD method					MCR method				
	TF	BN	MTC	NMT	MP	TF	BN	MTC	NMT	MP
Linearity										
Range ($\mu g\ mL^{-1}$)	12–1	0.5–15	2–28	0.5–15	0.5–10	1–12	0.5–15	2–28	0.5–15	0.5–10
Slope	1.9781	4.8289	1.0687	4.8249	1.9835	0.8665	8.1095	5.1362	150.6880	2.8190
Intercept	0.3396	0.7554	1.2752	0.7961	0.2933	−0.2357	0.5565	40.4734	55.8279	0.1550
Correlation Coefficient (r^2)	0.9998	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999	0.9998	0.9999	0.9999
Accuracy (Mean)	99.64	99.58	100.51	99.56	99.64	99.72	100.03	100.02	99.86	100.39
Precision (%RSD)										
Repeatability[a]	1.009	1.193	1.273	1.131	1.087	1.047	1.203	1.181	1.230	1.208
Intermediate Precision[b]	1.118	1.281	1.315	1.201	1.213	1.138	1.307	1.242	1.361	1.222
LOD[c] ($\mu g\ mL^{-1}$)	0.333	0.167	0.667	0.067	0.167	0.333	0.167	0.667	0.167	0.167
LOQ[c] ($\mu g\ mL^{-1}$)	1	0.5	2	0.5	0.5	1	0.5	2	0.5	0.5

[a] The intraday and:
[b] The interday, average of three different concentrations repeated three times within day and three times in three successive days, respectively.
[c] LOD and LOQ values were calculated using the following equations; LOD = 3.3 × SD / slope, LOQ = 10 × SD / slope [23].

4.3. Methods application

4.3.1. Quadruple divisor spectrophotometric method (QD)

For determination of TF, different concentrations of TF in the range of 1–12 $\mu g\ mL^{-1}$ were divided by sum standard spectra of 6 $\mu g\ mL^{-1}$ each of BN, MTC, NMT and MP, as a quadruple divisor, then the first derivative of the resulted ratio spectra were obtained and the amplitude values at 256 nm, Fig. 7, were plotted against the corresponding concentrations, and the regression equation was calculated and found to be:

$$Y_1 = 1.9781C_1 + 0.3396, r_1 = 0.9998, \text{for TF}$$

where Y_1 is the amplitude value at 256 nm, C_1 is the concentration of TF in $\mu g\ mL^{-1}$ and r_1 is the correlation coefficient.

For determination of BN, MTC, NMT and MP, different concentrations in the range 0.5–15, 2–28, 0.5–15 and 0.5–10 $\mu g\ mL^{-1}$, respectively, were divided by a sum standard spectrum of the other four components each of 8, 4, 8 and 6 $\mu g\ mL^{-1}$, respectively, then the first derivative of the resulted ratio spectra amplitudes at 336.6, 288.4, 292 and 254 nm, respectively, Fig. 8-11, were plotted against the corresponding concentrations, and the regression equations were then obtained and found to be:

$$Y_2 = 4.8289\ C_2 + 0.7554 \quad r_2 = 0.9999 \text{ for BN}$$

$$Y_3 = 1.0687\ C_3 + 1.2752 \quad r_3 = 0.9998 \text{ for MTC}$$

$$Y_4 = 4.8249C_4 + 0.7961\ r_4 = 0.9999 \text{ for NMT}$$

$$Y_5 = 1.9835\ C_5 + 0.2933\ r_5 = 0.9999 \text{ for MP}$$

where Y_2, Y_3, Y_4 and Y_5 are the first derivative amplitude values, C_2, C_3, C_4 and C_5 are the concentrations in $\mu g\ mL^{-1}$, r_2, r_3, r_4 and r_5 are the correlation coefficients of BN, MTC, NMT and MP, respectively.

4.3.2. Mean centering of ratio spectra spectrophotometric method (MCR)

For application of the proposed MCR method, the absorption spectra of different concentrations of TF, BN, MTC, NMT and MP in the ranges of 1–12, 0.5–15, 2–28, 0.5–15 and 0.5–10 $\mu g\ mL^{-1}$, respectively, were recorded in the wavelength range of 215–350 nm and the amplitude values of the mean centered fourth ratio spectra, obtained after using the corresponding divisors, Table 1, were plotted against the corresponding concentration of each component to construct their corresponding calibration curves. Regression equations were obtained and found to be:

$$Y_6 = 0.8665\ C_6 - 0.2357 \quad r_6 = 0.9999 \text{ for TF}$$

$$Y_7 = 8.1095\ C_7 - 0.5565 \quad r_7 = 0.9999 \text{for BN}$$

$$Y_8 = 5.1362\ C_8 + 40.4734 \quad r_8 = 0.9998 \text{for MTC}$$

$$Y_9 = 150.6880\ C_9 + 55.8279 \quad r_9 = 0.9999 \text{for NMT}$$

$$Y_{10} = 2.8191\ C_{10} + 0.1550 \quad r_{10} = 0.9999 \text{for MP}$$

where Y_6, Y_7, Y_8, Y_9 and Y_{10} are the amplitude values of the mean

Table 3
Determination of TF, BN, MTC, NMT and MP in laboratory prepared mixtures by the proposed methods.

Mix. No.	TF			BN			MTC			NMT			MP		
	Taken $\mu g\ mL^{-1}$	%Recovery*		Taken $\mu g\ mL^{-1}$	%Recovery*		Taken $\mu g\ mL^{-1}$	%Recovery*		Taken $\mu g\ mL^{-1}$	%Recovery*		Taken $\mu g\ mL^{-1}$	%Recovery*	
		QD	MCR		QD	MCR		QD	MCR		QD	MCR		QD	MCR
1	4	100.98	101.32	4	100.78	101.21	4	98.52	99.53	4	99.96	98.63	4	100.28	100.63
2	10	99.87	100.01	5	100.92	100.92	5	99.34	98.93	5	100.64	101.16	5	101.78	101.64
3	12	98.22	98.45	0.5	98.18	97.98	2	100.13	101.01	0.5	98.17	100.24	0.5	99.86	98.86
4	12	101.25	99.39	3	100.46	100.54	3	102.02	100.92	3	101.82	101.84	3	98.87	100.04
5	10	100.75	101.48	2	101.35	99.79	2	99.57	98.18	2	99.23	100.09	2	98.42	98.03
6	12	99.58	100.65	2	99.25	98.92	2	100.19	99.35	2	101.18	99.296	2	100.39	100.43
Mean ± RSD %		100.11 ± 1.129	100.22 ± 1.170	Mean ± RSD %	100.16 ± 1.201	99.89 ± 1.251	Mean ± RSD %	99.96 ± 1.178	99.65 ± 1.118	Mean ± RSD %	100.17 ± 1.334	100.21 ± 1.175	Mean ± RSD %	99.93 ± 1.197	99.93 ± 1.197

* Average of 3 determinations.

Table 4
Determination of Tolnaftate in pharmaceutical formulation by the proposed methods with application of standard addition technique.

Pharmaceutical formulations		Taken (μg)	% Recovery ± RSD	Standard addition technique[b]		
				Pure added (μg mL^{-1})	Found	% Recovery
Tinea Cure® Cream[a] B.N. 1,670,291 labeled to contain 1 g Tolnaftate/100 g	QD method					
	TF	4	93.97 ± 1.168	2	1.99	99.50
				4	4.05	101.25
				6	5.92	98.67
				Mean ± SD	99.81 ± 1.317	
	MCR method					
	TF	4	94.28 ± 1.394	2	2.03	101.50
				4	3.95	98.75
				6	5.92	98.67
				Mean ± SD	99.64 ± 1.611	

[a] Average of 6 determinations.
[b] Average of 3 determinations.

centered fourth ratio spectra at 285 nm, peak to peak 251–252 nm, 310–311 nm, 279 nm and peak to peak 312–313 nm for TF, BN, MTC, NMT and MP, respectively, C_6, C_7, C_8, C_9 and C_{10} are the concentrations in μg mL^{-1}, r_6, r_7, r_8, r_9 and r_{10} are the correlation coefficients of them, respectively.

4.4. Methods validation

The recommendations of the international conference on harmonization (ICH) [23] had been followed.

4.4.1. Range and linearity

Linearity of the proposed methods was evaluated and it was evident in the ranges of 1–12, 0.5–15, 2–28, 0.5–15 and 0.5–10 μg mL^{-1} for TF, BN, MTC and NMT, respectively, for QD method and 1–12, 0.5–15, 2–28, 0.5–15 and 0.5–10 μg mL^{-1} for TF, BN, MTC and NMT, respectively, for MCR method. Good linearity was evident from the high value of correlation coefficient as listed in Table 2. The concentration ranges and the other statistical parameters for the two proposed methods were listed in Table 2.

4.4.2. Accuracy

To assess the proposed methods accuracy, they were applied for determination of different blind TF, BN, MTC and NMT samples concentrations from the corresponding calculated regression equations and the results were recorded in Table 2. Accuracy was further checked by applying the standard addition technique on Tinea Cure® cream and good recoveries were obtained indicating no interference from excipients, as given in Table 4**.**

4.4.3. Precision

4.4.3.1. Repeatability. Three different concentrations each of the studied components were analyzed three times intra-day using the previously mentioned procedure under linearity of the proposed spectrophotometric methods. Good results, within the acceptable criteria, were obtained confirming good repeatability as given in Table 2.

4.4.3.2. Intermediate precision. The above mentioned procedures under repeatability were repeated inter-day on three different days for determination of the selected concentrations of TF, BN, MTC, NMT and MP. Acceptable RSD % values were obtained as given in Table 2.

4.4.4. Specificity

Specificity of the proposed methods was evaluated by applying the procedures mentioned under linearity of each method on different synthetic laboratory prepared mixtures of the five studied components in different ratios and calculating percent recovery and standard deviation. Good assay results were obtained confirming specificity of the proposed methods, Table 3.

4.4.5. Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection and limit of quantitation were calculated for degradates and impurity, according to ICH guidelines [23]. Low values of LOD and LOQ of BN, MTC and NMT and were obtained, Table 2, indicating high sensitivity of the proposed methods.

The proposed QD and MCR spectrophotometric methods were applied for determination of TF in its pharmaceutical formulation, Tinea Cure® cream and the results obtained were acceptable with good recovery and low SD values. The validity of the methods was further assessed by applying the standard addition technique which confirmed the accuracy of the proposed methods, Table 4.

The results obtained by applying the proposed QD and MCR spectrophotometric methods for determination of TF in Tinea Cure® cream were statistically compared to those obtained by applying the reference one [1] using Student's-t and *F*-tests where no significant difference was found between them regarding both accuracy and precision, Table 5. One way ANOVA test was performed for comparing between the developed QD and MCR spectrophotometric methods for determination of TF. No significant difference between them was observed as shown in Table 6.

Table 5
Statistical comparison between the proposed and the reference methods results for determination of Tolnaftate in its pharmaceutical formulation.

Items	Proposed QD method	Proposed MCR method	Reference spectrophotometric [1][b] method
Mean	93.97	94.28	94.13
SD	1.098	1.314	1.336
% RSD	1.168	1.394	1.419
n	8	8	8
Student's *t*-test (2.144)[a]	0.262	0.217	
F-value (3.787)[a]	1.480	1.034	

[a] Figures between parentheses represent the corresponding tabulated values of t and F at $P = 0.05$.
[b] Spectrophotometric determination of Tolnaftate at zero order absorbance maximum at 257 nm using methanol as a solvent.

Table 6
Results of ANOVA (one way) for comparison between the proposed methods and the reported ones **[1]** for determination of Tolnaftate (TF) in its pharmaceutical formulation.

Source of variation	TF					
	SS	df	MS	F	*P*-value	F crit
Between groups	0.369	2	0.185	0.117	0.900	3.4668
Within groups	33.014	21	1.572			
Total	33.383	23				

5. Conclusion

This work provided an insight into alkaline degradation pathway and degradates of TF then elucidation of their structures using IR, H1NMR and MS spectroscopy. The proposed quaternary divisor and mean centering of ratio spectra spectrophotometric methods were precise, accurate, reproducible, cost effective and specific and they can be used for the routine analysis of Tolnaftate in pure form and in its pharmaceutical formulation without interference from commonly encountered excipients. It is the first time to introduce such methods for assaying Tolnaftate and its related substances (degradation products and toxic impurities) with high sensitivity. Also, TF could be determined in its pharmaceutical formulation without interference from MP, that confirmed the high specificity of the proposed methods and recommended their use in quality control analysis of Tolnaftate.

Declaration of Competing Interest

No conflict of interest to declare.

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References

[1] BP, The British Pharmacopoeia, Her Majesty's, The Stationary Office, London, 2007.
[2] Martindale, The complete drug reference, 35th ed. Pharmaceutical Press, London, 2007.
[3] F.N. Marzulli, H.I. Maibach, Dermatotoxicology, fourth ed. Hemisphere Publishing Corp, New York, 1991 864.
[4] J.B. Sullivan Jr., G.R. Krieger (Eds.), Hazardous Materials Toxicology-Clinical Principles of Environmental Health, Williams and Wilkins, Baltimore, MD 1992, p. 1098.
[5] P.Y. Khashaba, S.R. El-Shabouri, K.M. Emara, A.M. Mohamed, Analysis of some antifungal drugs by spectrophotometric and spectrofluorometric methods in different pharmaceutical dosage forms, J. Pharm. Biomed. Anal. 22 (2000) 363–376.
[6] S.T. Patil, I.C. Bhoir, A.M. Bhagwat, M. Sundaresan, Assay of Tolnaftate and related impurities by isocratic supercritical fluid chromatography, Fresen. J. Anal. Chem. 367 (2000) 91–93.
[7] National Center for Biotechnology Information, PubChem Compound Database, CID=69675 https://pubchem.ncbi.nlm.nih.gov/compound/69675, Accessed date: 30 July 2018.
[8] C.S.P. Sastry, B.S. Sastry, J.V. Rao, R. Krishna, Spectrophotometric methods for the determination of Tolnaftate, Talanta 40 (1993) 571–576.
[9] T. Tahssen, N.R. Ahmed, Indirect spectrophotometric determination of Tolnaftate in pharmaceutical preparations, J. Educ. Sci. 22 (2009) 9–18.
[10] N. Bhoyar, T.K. Giri, A. Alexander, D.K. Tripathi, K. Ajazuddin, Development of UV spectrophotometric method for qualitative and quantitative estimation of Tolnaftate in different formulations, Res. J. Pharm. Technol. 5 (2012) 207–209.
[11] A.K. Dash, A liquid chromatographic method for the determination of Tolnaftate in pharmaceutical formulations, J. Pharm. Biomed. Anal. 11 (1993) 847–853.
[12] S.S. Zarapkar, K. Athalye, Reversed phase HPLC determination of Tolnaftate in cream and topical solution, Ind. J. Pharm. Sci. 52 (1990) 16–17.
[13] T. Kezutyte, O. Kornysova, A. Maruska, V. Briedis, Assay of Tolnaftate in human skin samples after in vitro penetration studies using high performance liquid chromatography, Acta Pol. Pharm. 67 (2010) 327–334.
[14] S. Sheikh, S. Asghar, S.A. Patni, Liquid chromatographic technique for stability indicating analytical method development and validation of salicylic acid and Tolnaftate in pharmaceutical ointment by high performance, Int. J. Sci. Res. Pub. 2 (2012) 250–253.
[15] C.N. Saha, S. Bhattacharya, A validated simultaneous RP-HPLC method for determination of betamethasone dipropionate and Tolnaftate in combined semisolid formulation, Int. J. ChemTech Res. 1 (2009) 671–674.
[16] M. Bapna, J.P. Balubhai, Development and validation of analytical method for simultaneous estimation of betamethasone dipropionate, Tolnaftate, Iodochlorhydroxyquin in pharmaceutical dosage form, Pharm. Chem. J. 2 (2015) 17–21.
[17] ICH Q1A (R2), Stability testing of new drug substances and products, Proceeding of the International Conference on Harmonization, 2003.
[18] K.C. Waterman, et al., Pharm. Dev. Technol. 7 (2002) 113–117.
[19] U.R. Kalkote, A.R. Choudhary, A.A. Natu, R.J. Lahoti, N.R. Ayyangar, Synth. Commun. (1991) 1889.
[20] N.W. Ali, N.S. Abdelwahab, M. Abdelkawy, A.A. Emam, A comparative study of ICH validated novel spectrophotometric techniques for resolving completely overlapping spectra of quaternary mixtures, Spectrochim. Acta, Part A 154 (2016) 114–122.
[21] M.M. Issa, R.M. Nejem, A.M. Abu shanab, N.T. Shaat, Resolution of five-component mixture using mean centering ratio and inverse least squares chemometrics, Chem. Cent. J. 7 (2013) 152–165.
[22] N.S. Abdelwahab, N.W. Ali, M. Abdelkawy, A.A. Emam, Validated RP-HPLC and TLC-densitometric methods for analysis of ternary mixture of cetylpyridinium chloride, chlorocresol and lidocaine in oral antiseptic formulation, J. Chromatogr. Sci. 54 (2015) 318–325.
[23] Validation of Analytical Procedures, Proceedings of the International Conference on Harmonization, Geneva. , ICH, 2005 Q2 (R1).