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Investigation into the Formation of the Genotoxic Impurity Ethyl Besylate in the Final Step Manufacturing Process of UK-369,003-26, a Novel PDE5 Inhibitor

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Abstract:

Sulfonate esters have a demonstrated potential for genotoxicity, and therefore their potential presence at trace levels in active pharmaceutical ingredients (APIs) has recently raised concerns [*Mesylate Ester Type Impurities Contained in Medicinal Products*; Swissmedic Department for Control of the Medicinal Products Market, 23rd October 2007 and Hoog, T. J.-d. *Request to Assess the Risk of Occurrence of Contamination With Mesilate Esters and Other Related Compounds in Pharmaceuticals*; Coordination Group for Mutual Recognition-Human Committee (CMDh), EMEA/CMDh/98694/2008: London, 27 February, 2008]. Sulfonate salts however, offer useful modification of physicochemical properties of active pharmaceutical ingredients (APIs) containing basic groups such that their use can at times offer significant advantages over other counterions [Elder, D. P.; Delaney, E.; Teasdale, A.; Eyley, S.; Reif, V. D.; Jacq, K.; Facchine, K. L.; Oestrich, R. S.; Sandra, P.; David, F. The Utility of Sulfonate Salts in Drug Development. *J. Pharm. Sci.* 2010, *99*, 2948−2961; DOI: 10.1002/jps.22058]. Indeed, the choice of benzenesulfonic acid as the counterion for the UK-369,003 API afforded many advantages over other salts such as citrate, hydrochloride, tartrate, and phosphate as well as other sulfonate salts such as tosylate, camsylate, and mesylate. The manufacturing route to the API consists of two C−C bond-forming steps (steps 1 and 2/Scheme 1) and a final salt-formation step (step 3/Scheme 1). The step 2 cyclisation process involves the use of ethanol as the reaction solvent. Residual levels of ethanol in the isolated product of the step 2 process was initially thought to be responsible for the formation of low levels of the genotoxic impurity ethyl besylate (ppm levels) during the final step salt-formation process [Glowienke, S.; Frieauff, W.; Allmendinger, T.; Martus, H. J.; Suter, W.; Mueller, L. *Mutat Res.* 2005, *581*, 23−34]. This was thought to result from subsequent reaction of residual ethanol with benzenesulfonic acid used in the final step (step 3). On the basis of this mechanistic hypothesis, the levels of residual ethanol in the isolated product from step 2 were controlled so that formation of ethyl besylate would be minimised or avoided in the final step. Spiking experiments coupled with deuterium labelling studies have shed doubt on this mechanism of formation. Our experimental results indicate that levels of ethyl besylate in the API are independent of the level of residual ethanol in the step 2 product (UK-369,003 free base) and are detected when higher than stoichiometric amounts of benzenesulfonic acid are used in the salt-formation process (step 3). This is thought to be due to a reaction between the excess benzenesulfonic acid and the ethoxy side chain of the API. Sensitive and selective analytical methods were also developed to detect and quantify subppm and higher levels of ethyl besylate and deuterated analogues.

1. Introduction

The use of sulfonic acids as suitable counterions for active pharmaceutical ingredients (APIs) has increased significantly over the past decade and now stands at 10% of the total cationic counterions that are being developed. The increase in the usage of such strong acids is at times ascribed to the general decrease in the aqueous solubility of new drug candidates.[5,6] In general, APIs that exhibit low melting points often exhibit plastic deformation during secondary processing which can cause both caking and aggregation, thus impacting both flow and compressibility performance.[7] Sulfonate salts typically produce higher-melting point versions of the API compared to those of other salts of the API, which in addition to processing advantages can enhance stability.[8]

UK-369,003 was nominated for development as the lead candidate for treatment of benign prostatic hyperplasia (BPH). The free base was found to be moderately crystalline with a melting point of 168 °C. Solubility of the free base at physiological pH was found to be poor hence necessitating a

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(1) *Mesylate Ester Type Impurities Contained in Medicinal Products*; Swissmedic Department for Control of the Medicinal Products Market, 23rd October **2007**.

(2) Hoog, T. J.-d. *Request to Assess the Risk of Occurrence of Contamination With Mesilate Esters and Other Related Compounds in Pharmaceuticals*; Coordination Group for Mutual Recognition-Human Committee (CMDh), EMEA/CMDh/98694/2008: London, 27 February, 2008.

(3) Elder, D. P.; Delaney, E.; Teasdale, A.; Eyley, S.; Reif, V. D.; Jacq, K.; Facchine, K. L.; Oestrich, R. S.; Sandra, P.; David, F. The Utility of Sulfonate Salts in Drug Development. *J. Pharm. Sci.* **2010**, *99*, 2948−2961; DOI: 10.1002/jps.22058.

(4) Glowienke, S.; Frieauff, W.; Allmendinger, T.; Martus, H. J.; Suter, W.; Mueller, L. *Mutat Res.* **2005**, *581*, 23–34.

(5) (a) Serajuddin, A. T. M. Salt Formation to Improve Drug Solubility. *Adv. Drug.Delivery.Rev.* **2007**, *59*, 603–616. (b) Elder, D. P. *J. Pharm Pharmacol.* **2009**, *61*, 269–278.

(6) Serajuddin, A. T. M.; Pudipeddi, M. In *Handbook of Pharmaceutical Salts; Selection and Use (International Union of Pure and Aapplied Chemistry*; Stahl, P. H., Wermuth, G., Eds.; Wiley-VCH: NewYork, 2002; Chapter 6: Salt-Selection Strategies. pp 135−160.

(7) Lee, S.; Hoff, C. In *Handbook Of Pharmaceutical Salts: Properties, Selection and Use (International Union of Pure and Applied Chemistry*; Stahl, P. H., Wermuth, G., Eds.; Wiley-VCH: New York, 2002; Chapter 8: Large Aspects of Salt Formation: Processing of Intermediates and Final Products. pp 191−220.

(8) Elder, D. P. Physicochemical and Crystallographic Investigations into the Salt Formation of Two Heterocyclic Drugs. *Ph.D. Thesis*; University of Edinburgh: UK, 1992.

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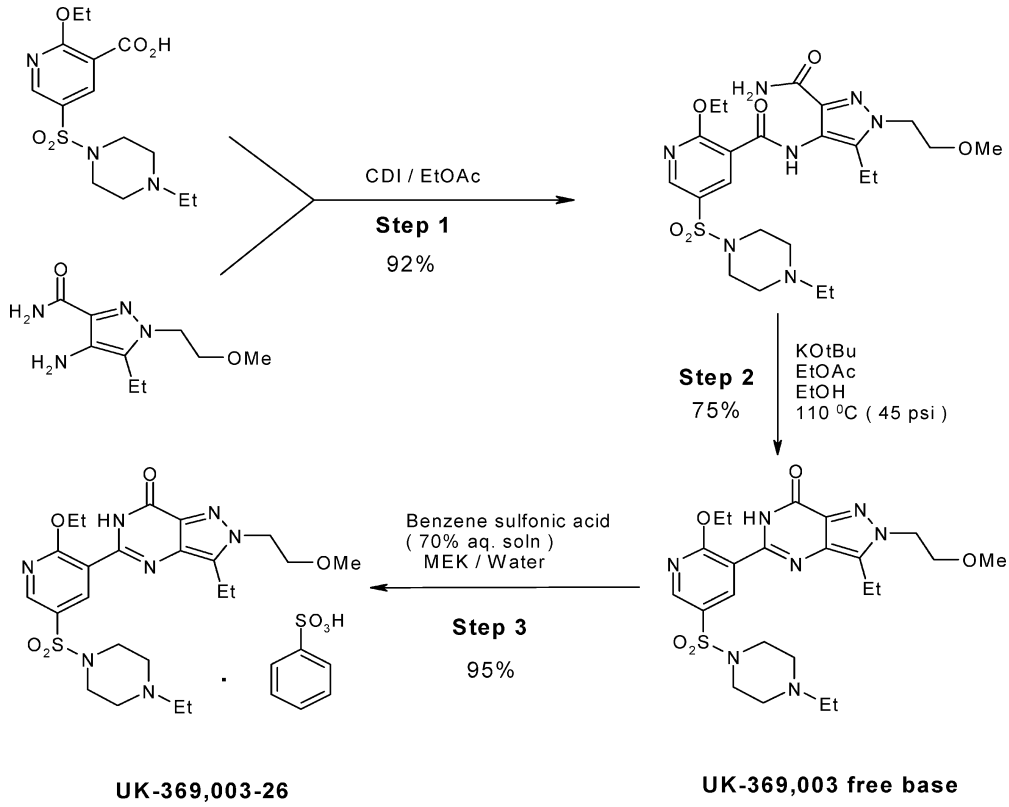
Scheme 1. **Manufacturing route to UK-369,003-26**[a]



[a] CDI = carbonyl diimidazole; MEK = methyl ethyl ketone; EtOAc = ethyl acetate; KOtBu = potassium tertiary butoxide; EtOH = ethanol.

comprehensive screen for a suitable salt form of the API. Benzenesulfonic acid was found to form the most suitable counterion for the API with a melting point of 248 °C and satisfied all our requirements for primary and secondary processing. The process for the formation of the benzenesulfonic acid salt involved the use of water/methyl ethyl ketone (4% water by volume) as the reaction medium. The water level at 4% ensured an optimum balance between product quality (purging of impurities) and the reaction yield. The cyclisation reaction (step 2/Scheme 1) involves the use of ethanol as the reaction media. Any residual amount of ethanol in the isolated step 2 product was therefore considered to be a considerable risk factor in the potential formation of ethyl besylate during the final step processing (step 3/Scheme 1).

The literature on the formation of sulfonate esters from interaction between sulfonic acids and alcohols is sparse, whilst there is much literature on the solvolytic instability of sulfonate esters.[9−13] Teasdale and co-workers demonstrated methanol C−O bond cleavage of isotopically labelled methanol (^{18}O-label) in its reaction with methanesulfonic acid leading to the formation of low levels of methyl methanesulfonate.[14] This is consistent with reversal of well established mechanisms for solvolysis of sulfonate esters. Their studies of various reaction profiles led to quantification of the levels of sulfonate ester formed under conditions relevant to the formation of methanesulfonate salts of pharmaceutically active bases. Their work demonstrated a clear scope to select conditions for the preparation of sulfonate salts in alcoholic solvents to minimize formation of sulfonate esters. Incorporation of water into the reaction system as well as eliminating excesses of sulfonic acid used are considered vital in avoiding formation of the sulfonate ester.[14]

Water was indeed incorporated in the process for the manufacture of the API (step 3/Scheme 1) and charge of the benzenesulfonic acid was not allowed to exceed the stoichiometric level. Subsequent to development of a reliable methodology for determination of residual ethyl besylate in isolated API (UK-369,003-26), 22 batches of API were tested for ethyl besylate. All batches showed levels <1 ppm. The level of residual ethanol in the free base (UK-369,003) leading to these batches of API was evaluated to be no more than 200 ppm. Our assumption was therefore such that a batch of free base UK-369,003 containing <200 ppm ethanol would lead to API with <1 ppm ethyl besylate. A maximum limit of 1 ppm ethyl besylate in API was considered to be our desired internal

(9) Winstein, S.; Grunwald, E.; Jones, H. W. *J. Am. Chem. Soc.* **1951**, *73*, 2700.
(10) Robertson, R. E. *Can. J. Chem.* **1953**, *31*, 589.
(11) Smith, M. B.; March, J. *March's Advanced Organic Chemistry*; Wiley: New York, 2001; p 464.
(12) Isaacs, N. *Physical Organic Chemistry*; Prentice Hall: Harlow, 1995; p 418.
(13) Bentley, T. W.; Bowen, C. T.; Brown, H. C.; Chloupek, F. *J. Org. Chem.* **1981**, *46*, 38.
(14) Teasdale, A.; Eyley, S. C.; Delaney, E.; Jacq, K.; Taylor-Worth, K.; Lipczynski, A.; Reif, V.; Elder, D. P.; Facchine, K. L.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *Org. Process Res. Dev.* **2009**, *13*, 429–433.

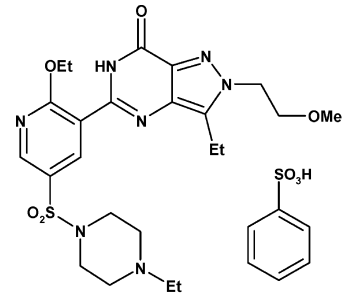
specification for this genotoxin. It is also worth noting that a 'threshold of toxicological concern' (TTC) approach which is stated by the European Medicines Agency (EMEA) would limit the daily patient exposure to no more than (NMT) 1.5 μg/day for supporting a market application (i.e., 15 ppm).[15]

Higher than 1 ppm ethyl besylate was detected in some of our small-scale laboratory batches as well as one pilot-plant batch of API. In studying the impact of using excess benzenesulfonic acid (>1 equiv) in the step 3 reaction, it soon became evident that this leads to API with >1 ppm ethyl besylate. More noteworthy was the fact that the in-going free base UK-369,003 did not contain residual ethanol at >200 ppm.

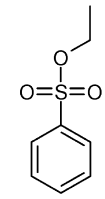
Further studies to investigate the cause for the formation of ethyl besylate began to shed doubt on the rationale of our initial hypothesis. Our findings led us to focus on the stoichiometry of the benzenesulfonic acid used in the step 3 salt-formation process.

The challenges presented in developing trace analytical methodology have been well documented. In particular matrix effects can have significant impact on the ability of the analyst to provide accurate and precise results robustly. These matrix effects can raise issues around recovery, precision, contamination, interference, and the legitimacy of results. To reduce these potential matrix effects it is necessary to remove the matrix at a stage in the analysis before the effects become active.

UK-369003 presented such challenges from the outset of method development where a simple, large-volume injection (LVI) gas chromatographic analysis with mass spectroscopic detection (GC/MS) was employed. Although the analysis appeared to proceed normally, positive results were obtained far in excess of those possible from the residual ethanol present in the batch. It was theorised that the matrix (API or impurity other than ethanol), was producing the ethyl besylate in the GC inlet during the desolvation and volatilisation process. A method developed in our laboratory that is generic for alkylators[16] and excludes the matrix from the chromatographic system was employed. The method utilises pentafluorothiophenol as a derivatising agent which is readily alkylated into a headspace (HS) and GC amenable species, with enhanced MS detectability. Although this form of sample pretreatment goes some way to reduce matrix effects, the test sample is heated in the HS oven with caustic and reactive reagents and cannot therefore be called universally applicable. UK-369003-26 produced positive results with this method, and a milder extraction procedure was sought. Liquid−liquid extraction has been well utilised in trace analysis and is still widely applied despite more automatable alternatives being available, e.g. solid-phase extraction (SPE), solid-phase micro-extraction (SPME), and stir-bar sorptive extraction (SBSE). It can provide a quick solution to method development where there is sufficient aqueous solubility and affinity of the matrix with sufficient lipophilicity of the target. It can also be a relatively quick technique to employ as equilibration/extraction times can be short relative to SPME or SBSE.



UK-369,003-26



Ethyl besylate

2. Experimental Section

2.1. General Procedure for the Synthesis of API (UK-369,003-26). To a reaction vessel was charged free base UK-369,003 (50 g, 0.096 mol). To this was added a mixture of methyl ethyl ketone (480 mL, 9.6 mL/g) and water (13.15 mL, 0.26 mL/g). The reaction mixture was heated to 70 °C over a period of 30 min to achieve dissolution. Benzenesulfonic acid solution (70% (w/w); 21.75 g, 0.1 mol) was added to the hot solution in four equal portions at hourly intervals. Product crystallisation was observed at the end of the first hour. The reaction was cooled to 0 °C at a rate of 0.1 °C/min. It was then further granulated at 0 °C for a period of 3 h. The white solid product was collected by filtration, and the wet cake was washed with methyl ethyl ketone (4 mL/g). The solid product was dried in a vacuum (50 °C) for a period of 12 h. The yield was in the order of 96%.

2.2. Analytical Method Development for Trace Levels of Ethyl Besylate. Due to a batch-dependent matrix effect, which was observed as variable recovery relative to external standards, the method utilized a standard addition approach. This was applied either as a dual level (zero and 1 ppm) limit test or multiple level (zero, 0.5 ppm, 1 ppm, and 2 ppm) addition for the purpose of validation.

Samples were dissolved in 0.1 M sodium hydroxide at 10 mg/mL; 0.5 mL of hexane or hexane containing ethyl besylate at appropriate spiking levels was added. The mixture was shaken and allowed to separate, and the upper organic layer was recovered for injection into a GC/MS. Experiments were performed, introducing the ethyl besylate spike as a methanolic solution into the aqueous test. These experiments showed that exposure of the spike to the test prior to extraction was not a significant factor in the determination. The ethyl besylate spike was therefore introduced in the extraction solvent for ease of use.

GC Conditions. Agilent 6890 GCs with 5975 or 5973 MSD detectors were used. Thermo-Fisher Trace GC and DSQ II MS were also used.

Two columns were employed which gave acceptable chromatographic performance. Due to the selectivity of the extraction and detection no specificity issues were encountered with either configuration.

Column 1 was a VF-5MS (Varian Inc., model CP912092), 20 m × 0.1 mm i.d. × 0.2 μm film thickness.

Column 2 ZB-5MS (Phenomenex Ltd.), 20 m × 0.18 mm × 0.18 μm film.

The oven temperature program for column 1 was 40 °C held for 2 min, 30 °C/min to 300 °C and held for 1.33 min. PTV

(15) *Guideline on the Limits of Genotoxic Impurities*, CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, 28 June 2006.

(16) Alzaga, R.; Ryan, R. W.; Taylor-Worth, K.; Lipczynski, A.; Szucs, R.; Sandra, P. *J. Pharm. Biomed. Anal.* **2007**, *45*, 472–479.

injection at 30 °C held for 1 min then ramped to 250 °C at 500 °C/min. Injection volume was 5 μL, and the split flow was 100 mL/min for 1 min, splitless during the temperature ramp, and 30 mL/min after 2 min. SIM detection was used monitoring *m/z* 77, 141, and 186.

Column 2 used a split/splitless injector in splitless mode (50 mL/min split at 1.0 min), with a 1 μL injection. The inlet was maintained at 250 °C. The oven temperature program was 60 °C for 0.5 min then 40 °C/min to 250 °C. Column flow was 1.5 mL/min constant.

2.3. Analytical Performance. In the single step, extraction employed 75% of ethyl besylate partitioned into the organic phase. Limits of detection were ≪1 ppm. Injection repeatability ($n = 6$) typically gave less than 10% relative standard deviation (RSD), at the 1 ppm relative level. Repeatability in the presence of the test ($n = 3$, independent extractions) gave an RSD of 2%. Linearity with extracted external standards over the range 0.1−2 ppm gave a coefficient of determination r^2 of 99.9%. Linearity of ethyl besylate response over the spiking range 0−2 ppm (in a test containing 2 ppm ethyl besylate) gave an r^2 of 98.9%. Recovery against extracted standards was variable and between 15 and 120%, but as previously indicated this was compensated for by the application of a standard addition approach to the determination.

In order to understand the significance of the results, repeatability was performed for the analytical procedure and the experimental procedure including batch synthesis. Analytical precision gave RSDs of 1.4% ($n = 6$) for injection, and 15.7% ($n = 3$) for multiple test preparations and extractions. Experimental precision was obtained from the results of the determination of ethyly besylate in three batches of UK-369003 synthesised in parallel, using identical conditions and reagents. An RSD of 55% indicated that the variability present in chemical synthesis, which affected the actual ethyl besylate content of UK-369003 batches, was more significant than the analytical variability.

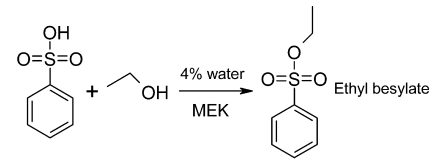
Overall the validation data demonstrated that the analytical procedure was suitable for the accurate and precise determination of ethyl besylate in UK-369003 but that care was required to compare batch results and to draw significance from trends.

Results and Discussion

Our initial mechanistic hypothesis that drove our plans for limiting the residual level of the ethyl besylate in the API was based on controlling the level of residual ethanol in the isolated free base UK-369,003. It was thought that a reaction between ethanol and benzenesulfonic acid during the course of the salt-formation process (step 3/Scheme 1) would lead to the formation of ethyl besylate.

Our laboratory experimentation (Scheme 2) did indeed show formation of low levels of ethyl besylate (4−6 ppm) over a period of 6 h and increased levels (>1000 ppm) over prolonged reaction time when ethanol (100 mol equiv) and benzenesulfonic acid (1 mol equiv) were refluxed in methyl ethyl ketone in the presence of low levels of water (~4% by volume). (The reaction condition used here was to mimic the step 3 salt-formation process.). Our data indicate that even in the presence of a large excess of ethanol under reflux conditions, only a relatively small amount of ethyl besylate is formed. This is in line with the findings reported by Teasdale and co-workers when low levels of methyl methanesulfonate were formed from interaction between methanol and methanesulfonic acid.[14]

Scheme 2[a]



Experiment	2 hrs reflux EtB ppm	4 hrs reflux EtB ppm	6 hrs reflux EtB ppm
A	4	5	6

[a] EtB = ethyl besylate; MEK = methyl ethyl ketone.

Table 1. **Step 3 salt-formation process**

experiment	ethanol spike (ppm)	ethyl besylate level in API (ppm)
control	111	0.18
A	500	0.06
B	10000	0.08

Table 2. **Step 3 process using an overcharge of BSA (2 equiv) and spiked with ethanol-d_6[a]**

experiment number	ethyl besylate (ppm) in API	ethyl besylate-d_5 (ppm) in API
A	1671	ND

[a] ND = none detected; BSA = benzenesulfonic acid.

In order to provide further supporting evidence that the presence of residual ethanol in the free base UK-369,003 is the main contributor towards the formation of the ethyl besylate during the step 3 salt-formation process, we carried out a series of spiking experiments.

Our current salt-formation process (step 3) was spiked with varying levels of ethanol (500 and 10,000 ppm), and the isolated API was tested for residual levels of ethyl besylate and compared with that of the control experiment. Our results indicated no obvious correlation between residual ethanol in UK-369,003 free base and ethyl besylate in the isolated API (Table 1).

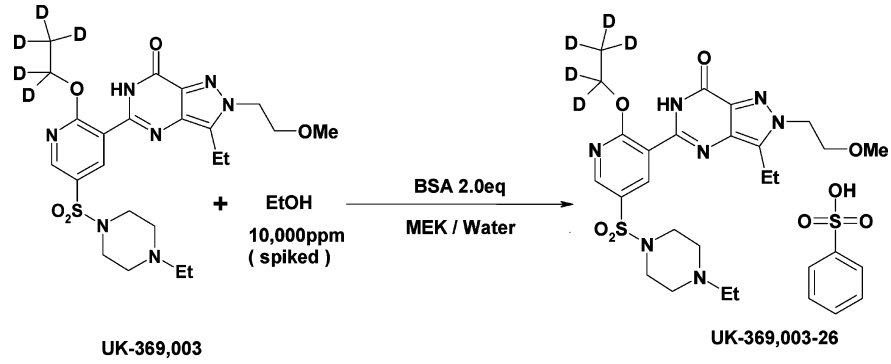
This finding placed doubt on the validity of our initial mechanistic hypothesis for the ethyl besylate formation.

We also investigated the effect of increasing the stoichiometry of the benzenesulfonic acid used in the salt-formation process whilst keeping other factors unchanged. The input UK-369,003 free base contained no residual ethanol. Our results indicated that an increase in the stoichiometry of benzenesulfonic acid (0.2 equiv) led to an increase (>1 ppm) in the residual level of detected ethyl besylate in the isolated API.

We needed to understand the origin of the ethyl moiety of the resultant ethyl besylate in the API.

If residual ethanol in the input UK-369003 free base plays no part in the formation of the ethyl besylate, the ingoing free base itself must therefore be involved in the formation of this impurity. In order to test this hypothesis a salt-formation process (step 3), with an overcharge of the acid spiked with ethanol-d_6, was carried out. It resulted in API with no detectable level of

Table 3

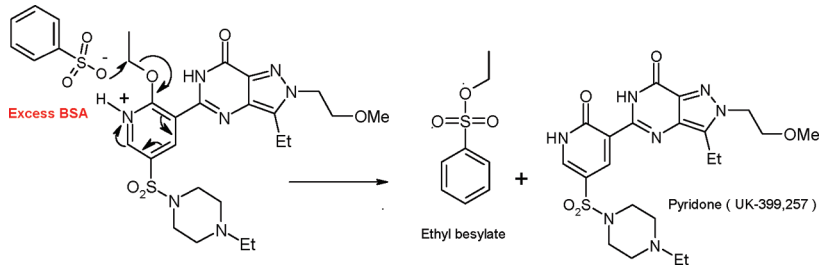


experiment number	ethyl besylate (ppm) detected in API	ethyl besylate-d_5 (ppm) detected in API
A	10	1100

ethyl besylate-d_5 but a significant level of ethyl besylate (1671 ppm) (Table 2).

In view of this result, we developed the following hypothesis as an explanation for the origin of the ethyl besylate. The ingoing free base UK-369003 can react with any excess (>1 equiv) benzenesulfonic acid, leading to the formation of the undesired ethyl besylate.

We therefore postulated the following mechanism:



Our results indicated that a slight excess of BSA (>1 equiv) charge could lead to the formation of undesirable levels of the ethyl besylate during the final step salt formation. It has to be noted that residual levels of pyridone (also formed in step 2 reaction and carried forward through to the step 3) are normally detected at slightly higher levels in API, and we consider this increase to be partly associated with our proposed mechanism shown above.

In an attempt to further verify our hypothesis, a deuterated analogue of UK-369003 (i.e., protons of the ethoxy side chain were substituted by deuterium) was subjected to the step 3 reaction process in the presence of excess acid (2 equiv) as well as residual ethanol (10,000 ppm) (Table 3).

Analysis of the isolated API showed the presence of a significant level of ethyl besylate-d_5 (1100 ppm) and a very low level of ethyl besylate (10 ppm) (^{1}H NMR of the ingoing deuterated UK-369003 showed the presence of a residual amount of the non-deuterated UK-369003. This in turn explains the presence of ethyl besylate at 10 ppm.)

This finding is consistent with our revised mechanism for the formation of ethyl besylate in the final step 3 salt-formation process.

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