Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1339–1344

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of an automated static headspace gas chromatography–mass spectrometry (SHS-GC–MS) method for monitoring the formation of ethyl methane sulfonate from ethanol and methane sulfonic acid

Karine Jacq[a], Ed Delaney[b], Andrew Teasdale[c], Steve Eyley[c], Karen Taylor-Worth[d], Andrew Lipczynski[d], Van D. Reif[e], David P. Elder[f], Kevin L. Facchine[g], Simon Golec[h], Rolf Schulte Oestrich[i], Pat Sandra[a], Frank David[a,*]

[a] *Research Institute for Chromatography, Pres. Kennedypark 26, B-8500, Kortrijk, Belgium*
[b] *Reaction Science Consulting LLC, Princeton, NY 08540, USA*
[c] *Astra Zeneca, R&D Charnwood, Bakewell Road, Loughborough, Leicestershire, LE11 5RK, United Kingdom*
[d] *Pfizer Global Research and Development, Analytical R&D, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom*
[e] *Schering-Plough, 556 Morris Avenue, Summit, NJ 07901-1330, USA*
[f] *GlaxoSmithKline Research and Development, Park Road, Ware, Hertfordshire, SG12 0DP, United Kingdom*
[g] *GlaxoSmithKline Research and Development, Five Moore drive, Research Triangle Park, NC 27709-3398, USA*
[h] *Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[i] *F. Hoffmann-La Roche Ltd., Grenzacher Strasse, 4070 Basel, Switzerland*

ARTICLE INFO

Article history:
Received 16 June 2008
Received in revised form 5 September 2008
Accepted 14 September 2008
Available online 27 September 2008

Keywords:
Ethyl methane sulfonate formation
Automated sample preparation
Static headspace
Derivatisation
GC–MS

ABSTRACT

An automated sample preparation and analysis procedure was developed to monitor the formation of ethyl methane sulfonate from reaction mixtures containing ethanol and methane sulfonic acid. The system is based on a liquid handling robot combined with a static headspace module. The formed ethyl methane sulfonate is analysed after derivatisation with pentafluorothiophenol using static headspace-gas chromatography–mass spectrometry (SHS-GC–MS).

Using the automated reaction–derivatisation–headspace GC–MS system, the formation of ethyl methane sulfonate can be monitored in different reaction mixtures under different reaction conditions, including temperature, water content and pH. Excellent linearity, repeatability and robustness were obtained, allowing the system to be used in kinetic studies.

1. Introduction

Sulfonic acids are widely used for salt formation during the synthesis and production of active pharmaceutical ingredients (APIs) [1]. In the presence of low molecular weight alcohols, such as methanol, ethanol or isopropanol, sulfonic acids can lead to the formation of corresponding sulfonates. These esters are considered as potential alkylating agents that may exert genotoxic effects in bacterial and mammalian cell systems [2], and therefore their potential presence as trace level impurities in active pharmaceutical ingredients (APIs) is a concern which needs to be appropriately managed and controlled as directed in recent regulatory guidances and communications [3,4]. In order to better understand the mechanisms and kinetics governing the formation of these sulfonate esters, a series of experimental studies has been initiated by a group of innovative multi-national pharmaceutical companies operating within the framework of the Product Quality Research Institute (PQRI). In a first stage, the formation of ethyl methane sulfonate (EMS) from methane sulfonic acid (MSA) and ethanol was studied. Therefore an analytical procedure was needed to monitor EMS in ethanol/MSA reaction mixtures and the developed method should allow the evaluation of different reaction conditions, including presence of water or bases, different pH, reaction temperature and reaction times.

For the determination of alkyl esters of sulfonic acids in APIs different methods have been developed and used, as described in a recent review by Elder et al [5]. Direct analysis of alkyl esters of methanesulfonates by gas chromatography (GC) was used by Ramijt et al. [6] and Li [7], respectively in combination with mass spectrometric (MS) and flame ionization (FID) detection. Although ppm (μg/g) sensitivity was obtained, direct injection of sulfonates

* Corresponding author. Tel.: +32 56 204031.
E-mail address: frank.david@richrom.com (F. David).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.09.028

in API matrix can lead to inlet contamination and/or solute degradation [7,8]. In addition, we also observed occasional formation of sulfonate esters in heated inlet systems (through sample pyrolysis and flash reaction with solvents). To avoid the introduction of non-volatile and reactive material in the GC inlet, extraction methods such as (micro-) liquid-liquid extraction, solid phase micro-extraction (SPME) and solid phase extraction (SPE) [9] were tested for selective extraction and enrichment of sulfonate esters. Extraction methods such as SPME are however restricted to aqueous API solutions (or aqueous reaction media).

As an alternative to gas chromatography, liquid chromatography (HPLC) methods have also been developed for the analysis of alkyl and aryl sulfonate esters [8]. Although thermal decomposition of the API is less likely to occur, reaction between alcohols (in the mobile phase or solvent) and trace levels of acids (present as impurities in the API or intermediate) could potentially lead to formation of sulfonate esters and consequently to false positive results. Moreover, the stability of sulfonate esters in aqueous solutions and mobile phases can be questioned.

To overcome problems with solute stability prior to and during analysis, esters of methane sulfonic acid were also determined by GC after derivatisation with sodium thiocyanate by Lee et al. [10]. The corresponding alkylthiocyanates and alkylisothiocyanates were analysed by static headspace (SHS) coupled to GC–MS. High sensitivity and acceptable repeatability were achieved. The major drawback of this method was the (slow) hydrolysis of alkyl mesylate esters in the aqueous reaction mixture. Recently, another derivatisation method was described by Alzaga et al. [11] allowing determination of methyl, ethyl and isopropyl esters of sulfonic acids in API's at sub-ppm level. The method was based on in-situ derivatisation using pentafluorothiophenol (PFTP), followed by static headspace and GC–MS analysis. This method could be applied to aqueous and non-aqueous (dimethyl sulphoxide) API solutions. For accurate quantification, corresponding internal standards were synthesized using deuterated alcohols. By derivatisation of the sulfonate esters with PFTP, the formation reaction is stopped and the static headspace sampling avoids contamination of the analytical system. Excellent sensitivity, linearity, repeatability and solute stability (of the derivatised solutes) were obtained, and therefore this method was used as a basis for the current study. However in contrast to the work of Alzaga et al. [11], this work did not focus on the determination of trace levels of EMS in API, but upon the formation of EMS from concentrated reaction mixtures. High precision and reproducibility over a wide linear dynamic range of the analytical method are thus required. The derivatisation-headspace GC–MS method was fully automated using a robotic system and applied to the analysis of methane sulfonic acid/ethanol reaction mixtures. The automated method and its validation in terms of linearity, repeatability and robustness are described in this paper. In addition, some examples of the monitoring of ethyl methane sulfonate formation in different reaction mixtures and different conditions are shown.

2. Experimental

2.1. Chemicals

Methane sulfonic acid (MSA), methane sulfonyl chloride (MSC), ethyl methane sulfonate (EMS), pentafluorothiophenol (PFTP), dimethyl sulfoxide (DMSO), 2,6-lutidine, di-isopropyl ethyl amine (Hunig's base) and ethanol (absolute, EtOH) were obtained from Sigma-Aldrich (Beerse, Belgium). Pentafluoroanisole (PFA), sodium sulfate (anhydrous) and sodium hydroxide were from Acros Organics (Thermo Fisher, Geel, Belgium) and d_6-ethanol (d_6-EtOH) was from Biosolve (Valkenswaard, NL)

2.2. Internal standard preparation

1 g methane sulfonyl chloride was mixed with 1 mL d_6-ethanol in a reaction tube, closed with a Teflon lined screw cap. The reaction mixture was heated for 72 h at 70 °C. After cooling, 2.5 mL water was added followed by 2.5 mL diethylether (CAUTION: volatile acidic vapours). The formed d_5-ethyl methane sulfonate (d_5-EMS) was extracted in the ether phase. This phase was separated, dried over sodium sulfate, concentrated under nitrogen and diluted in 10 mL acetonitrile (CAUTION: genotoxic material). The solution was stored at 4 °C. The exact concentration of the internal standard in this solution was checked by GC–MS using liquid injection and using EMS as external standard. The analytical conditions were similar to the conditions used for headspace analysis (see below). The use of methane sulfonyl chloride resulted in a much higher reaction yield and higher concentration of the deuterated internal standard than the previously described method using methane sulphonic acid [11].

2.3. Solutions

The following solutions were prepared:

- Reaction mixture: MSA was diluted at a typical concentration of 100 mg/mL (around 1.04 M) in ethanol. Bases or water can also be added to this reaction mixture. This reaction mixture is premixed and 1 mL aliquots are transferred to several 2 mL vials.
- Derivatization solution: mixture of pentafluorothiophenol (6.4 mg/mL) and sodium hydroxide (20 mg/mL) in water.
- Internal standard solution: mixture of 50 ng/μL pentafluoroanisole (IS 1) and 100 ng/μL d5-EMS (synthesized, IS 2) in acetonitrile.
- Dilution solvent in SHS vials: DMSO/H_2O (1:1).
- External standard solution for validation: EMS was diluted at different concentrations between 5 and 500 μg/mL in ethanol, acetonitrile or in reaction mixture (see above) for linearity and reproducibility tests.

2.4. GC–MS analysis

GC–MS analyses were performed on a Agilent 6890GC-5973MSD system (Agilent Technologies, Wilmington, DE, USA), equipped with a Gerstel dual rail MPS2 sampler (Gerstel GmbH, Mülheim, Germany). A schematic diagram of the sampler is shown in Fig. 1. The available vial trays were filled as follows:

- Tray C: 98 position temperature controlled tray for 2 mL reaction vials. The vials contain 1 mL reaction mixture (MSA in ethanol).
- Tray D: 32 position tray for 20 mL vials. The vials contain 2 mL DMSO/water (1:1 mixture).
- Tray E: 2 trays with each 5 mL and 10 mL vials containing IS solution, derivatisation reagent solution and wash solvents.

The typical sample preparation sequence is as follows:

- Transfer 20 μL reaction mixture from heated tray (Fig. 1, C) at time $t = x$ to 20 mL headspace vial (with 2 mL DMSO/water) in tray D.
- Add 20 μL IS solution (from E to D).
- Add 100 μL derivatisation solution (from E to D).
- Perform headspace analysis (using headspace syringe B and agitator/heater F).

Between the liquid sample handling steps, syringe washing is performed using the wash solvents in the E trays.

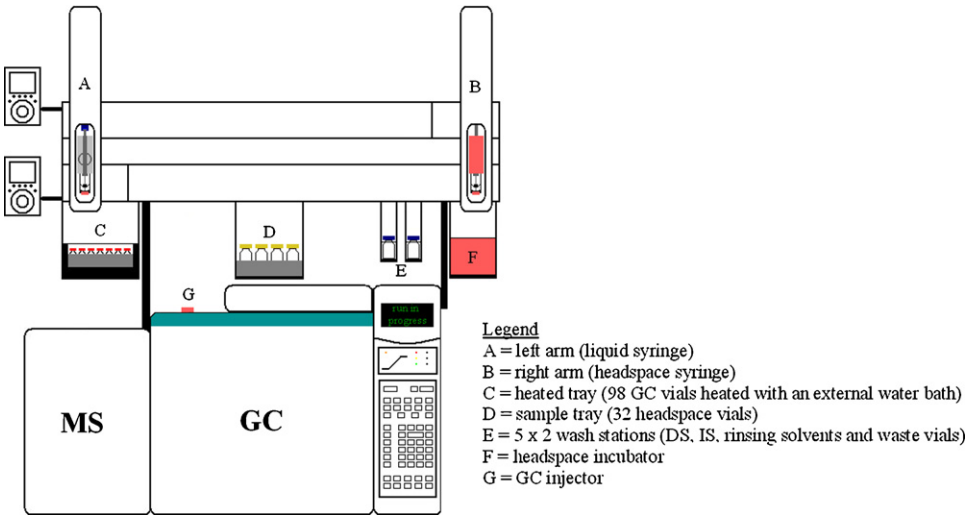

Fig. 1. Schematic diagram of dual rail robotic system for automated liquid handling, derivatisation and static headspace GC–MS analysis.

Static headspace equilibration was performed at 105 °C for 15 min, while shaking at 600 rpm. Injection of 1 mL headspace gas was done using a heated (110 °C) gastight syringe (2.5 mL) in split mode (1/10 split ratio) at 250 °C (split/splitless inlet temperature). Separation was performed on a 20 m × 0.18 mm i.d. × 1 μm df DB-VRX column (Agilent Technologies). Helium at 0.8 mL/min constant flow (125 kPa at 60 °C) was used as carrier gas. The oven was programmed from 60 °C (1 min) at 10 °C/min to 130 °C and at 30 °C/min to 250 °C. Detection was done by electron ionization MS in SIM mode. A solvent delay time of 3.5 min was used and the following ions were monitored:

3.5–6.3 min: 155, 183, 198 (pentafluoroanisole);
6.3–7.5 min: 79, 97, 109 (EMS), 111, 130 (EMS-d_5);
7.5–8.4 min: 199, 214 (Me-TPFB);
8.4–12.0 min: 200, 228 (Et-TPFB), 201, 233 (Et-TPFB-d_5).

Ions 198, 109, 111, 228 and 233 were used for the integration of respectively pentafluoroanisole, EMS, EMS-d_5, Et-TPFB, and Et-TPFB-d_5.

Transfer line temperature was 260 °C, source temperature was 230 °C and quadrupole temperature was 150 °C.

The reactions taking place in heated tray C (formation) and in the SHS incubator F (derivatization) are shown in Fig. 2.

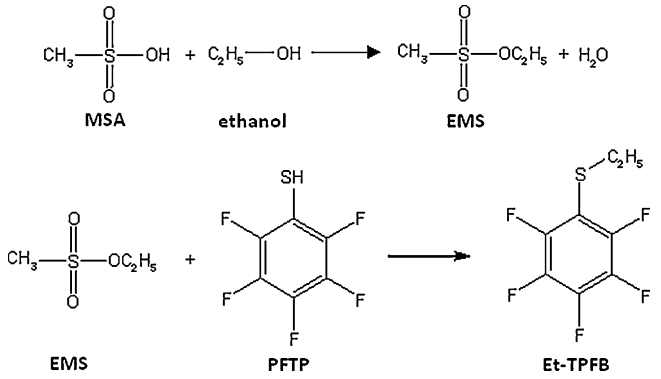

Fig. 2. Reaction mechanisms for EMS formation from methane sulphonic acid and ethanol (above) and EMS derivatisation reaction with pentafluorothiophenol (below).

3. Results and discussion

A typical chromatogram obtained for the analysis of a reaction solution containing 250 μg/mL EMS is shown in Fig. 3. The derivatised EMS elutes at 9 min as pentafluorophenyl-ethyl sulfide (Et-TPFB). The deuterated internal standard (Et-TPFB-d5) elutes just before Et-TPFB. Both peaks can be quantified using extracted ion chromatograms at m/z 233 and 228 respectively.

The peak for the pentafluoroanisole (PFA) elutes at 5.3 min. This internal standard, which has a chemical structure similar to the derivatised solutes (methylether instead of ethylsulfide) was used to monitor instrument performance. A large deviation observed on the peak area of PFA would indicate an error in IS addition (liquid handling) and/or in static headspace analysis.

In addition, the specific ions for EMS and d5-EMS are monitored in a time window between 6.3 and 7.5 min. This was done in order to detect underivatised EMS or internal standard. These compounds

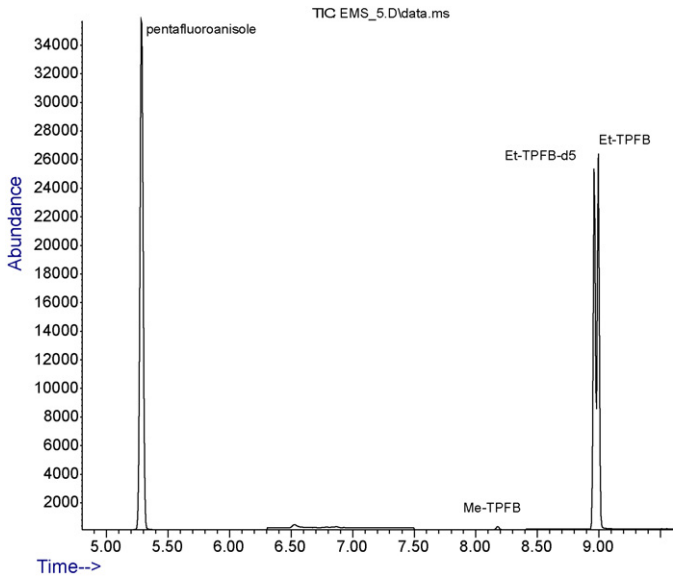

Fig. 3. Chromatogram obtained by static headspace GC-(SIM)MS analysis of MSA/ethanol reaction mixture spiked with EMS. (Me-TPFB is a trace impurity of derivatised methyl methanesulphonate, ET-TPFB-d5 and Et-TPFB are respectively the derivatised internal standard and derivatised EMS).

Table 1
Validation of derivatisation-SHS-GC–MS method.

EMS (μg)	PFA	Me-TPFB	Et-TPFB d5	Et-TPFB	Rel Area
0	280134	2016	200163	333	0.002
5	300742	7741	223447	5937	0.027
5	294712	1922	222579	6252	0.028
5	307885	2030	229717	6421	0.028
25	286297	1921	209304	26984	0.129
50	329687	2192	245241	61758	0.252
50	330967	6160	248046	62180	0.251
50	328756	2117	244613	61550	0.252
125	339988	3245	248132	163159	0.658
275	335488	2396	129735	179660	1.385
500	317337	4587	228441	582145	2.548
RSD all	14.3		15.5		
RSD 5 μg	2.2		1.7	4.0	3.1
RSD 50 μg	0.3		0.7	0.5	0.2
Slope					0.00509
Intercept					0.00138
R^2					0.99988

The table shows the raw peak areas for IS1 (column 2), for MMS derivative (column3), for IS2 and EMS derivatives (columns 4 and 5) and the relative peak area (Et-TPFB versus IS2) (column 6) in function of EMS concentration spiked in reaction mixture, at room temperature (column 1). Relative standard deviations (RSDs) at all levels and at 5 μg/mL and 50 μg/mL, and linearity data are given. EMS and d_5-EMS were not detected.

are not detected in Fig. 3, indicating that the derivatisation reaction was complete.

Finally, the derivatised MMS (Me-TPFB) is monitored in the time window between 7.5 and 8.4 min. As already observed by Alzaga et al. [11], a small trace of this derivative is observed, even in blank samples.

Method validation was performed using 1 M solutions of MSA in ethanol spiked with EMS and placed in tray C at room temperature. The concentration of the EMS was in the range of 5–500 μg/mL. This range corresponds to a 0.005% to 0.5% (potential) conversion of MSA into EMS. Using the spiked solutions, a six level (+ blank) calibration curve was made. The repeatability at 5 μg/mL and at 50 μg/mL EMS levels were measured. The results are summarized in Table 1.

The table shows the raw peak areas for pentafluoraoanisole (PFA, IS1) (column 2), for the MMS derivative (column3), for IS2 (deuterated derivatised EMS) (column 4) and EMS derivative (column 5) and the relative peak area (Et-TPFB versus IS2) (column 6) in function of EMS concentration spiked in reaction mixture. Relative standard deviations (RSDs) at all levels and at 5 μg/mL and 50 μg/mL, and linearity data are given. EMS and d_5-EMS were not detected.

From these data, it is clear that the standard deviation of internal standard 1 is of the order of 15% (absolute peak area for PFA) reflecting the instrumental variations. The relative standard deviation for

Table 2
Linearity of derivatisation–SHS-GC–MS method for MSA/ethanol reaction mixtures spiked with EMS and thermostated at 70 °C.

EMS (μg)	PFA	Et-TPFB d_5	Et-TPFB	Rel area
0	272620	214256	39293	0.18
50	296782	236358	99604	0.42
100	231752	179151	116711	0.65
250	235959	180091	264580	1.47
375	289672	224627	457359	2.04
500	210138	156311	410532	2.63
RSD	13.7	15.7		
Slope				0.00495
Intercept				0.18042
R^2				0.9991

Table 3
Stability of calibration curves.

Curve	Slope	Intercept	R^2
1 (Table 1)	0.00509	0.001	0.999
2	0.00514	0.048	0.998
3	0.00514	0.024	0.998
4	0.00494	0.020	0.998
5 (70 °C - Table 2)	0.00495	0.180	0.999
Mean	0.00501		
s	0.00013		
RSD	2.49		

The table compared the linearity data obtained for four independent series of tests using spiked reaction mixtures at room temperature and one series of spiked reaction mixtures at 70 °C.

EMS (as Et-TPFB derivative), measured relative to the internal standard 2 (d_5-Et-TPFB) was 3% at 5 μg level and better than 1% at the 50 μg level. The linearity was also excellent when using internal standardization. The role of the internal standard is clearly illustrated by the calibration point at 275 μg (spiked amount of EMS). The peak area obtained for Et-PTFB is too low, but the corresponding lower response of the internal standard corrects for this.

The limit of detection (LOD) for the determination of EMS in the MSA/ethanol reaction mixture is lower than 0.5 μg/mL. In the "blank" mixture (not spiked, 0 level), however, also a small trace of EMS (detected as Et-TPFB) is measured. The limit of quantification (LOQ), as derived from the calibration curve is 1 μg/mL, corresponding to 0.001% conversion. This value is more than sufficient for the reaction monitoring purpose.

Next, both linearity and repeatability were tested using heated (70 °C) MSA/ethanol mixtures spiked with EMS. The results are

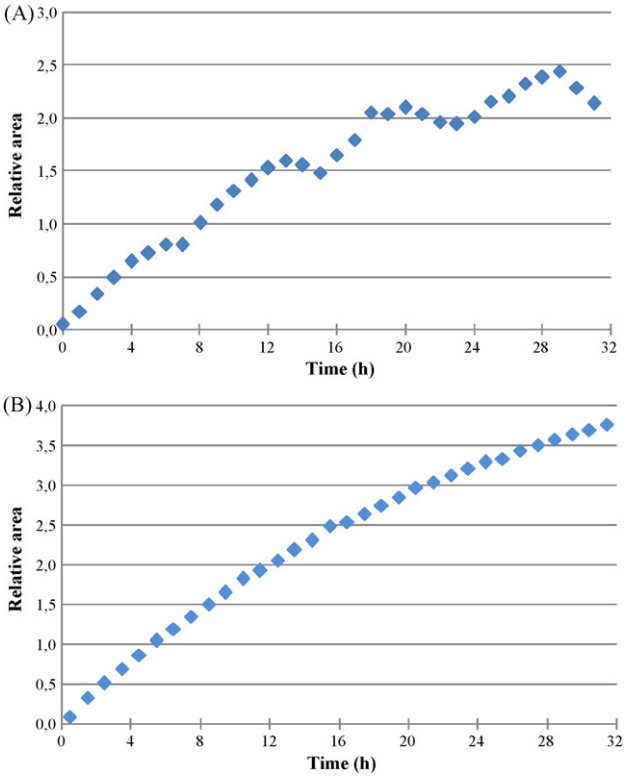

Fig. 4. Plot of EMS formation under anhydrous conditions at 70 °C as a function of time. Plot A shows the relative peak area (EMS versus IS, both as TBPB derivatives) obtained using a 32 position 10 mL vial tray with block heating. Plot B shows the same data obtained using a 98 position 2 mL vial tray heating with a circulating water bath.

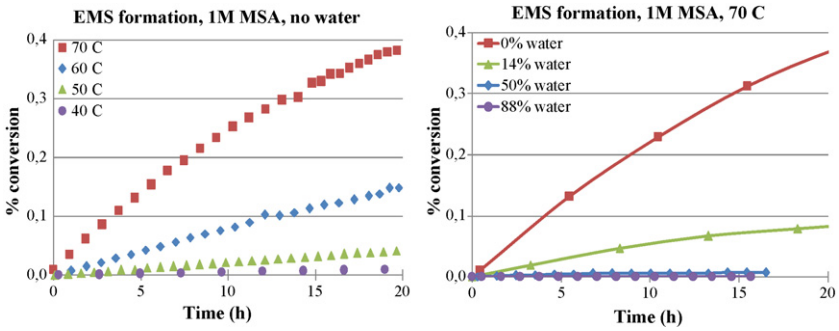

Fig. 5. Plot of EMS formation as a function of time under different reaction conditions. (A, left) Anhydrous, different temperatures; (B, right) 70 °C, different water concentrations.

shown in Table 2. Again a good linearity is obtained. In this case, however, EMS (as Et-TPFB) was also detected in the non-spiked (0 level) sample. This indicates that EMS was formed from MSA and ethanol (in the heated tray at 70 °C). The analysis of the non-spiked mixture (0 level) and the spike at 250 μg/mL was repeated three times and relative standard deviations were respectively 3.5% and 2.2%.

The slope of this calibration curve (0.00495) was similar to the slope for the test mixture in acetonitrile (0.00509), indicating that ethanol does not interfere in EMS measurement. The precision of the transfer of 20 μL reaction mixture from tray C is not influenced by solvent choice (ethanol or acetonitrile) or by reaction vial temperature (room temperature or 70 °C). Also in these tests, neither of the starting materials (EMS nor EMS-d_5) were detected, confirming that the derivatisation reaction was complete. Here also the deviation of the absolute peak areas on the highest calibration level (500 μg) is corrected by the deuterated internal standard.

Finally, three additional calibration curves were prepared on three different days in order to evaluate robustness and instrument stability. The characteristics of the three curves are summarized in Table 3 and compared to the curves given in Tables 1 and 2. Slope, intercept and correlation coefficients (R^2) are given in the table for 4 independent series of tests using spiked reaction mixtures at room temperature and one series of spiked reaction mixtures at 70 °C. Each time good linearity was obtained and the standard deviation on the slopes was very small (2.5%). The stability of the calibration curves and responses at the calibration levels indicate excellent day-to-day precision.

From these results it was concluded that sensitivity, repeatability, linearity and robustness were sufficient to apply the automated method for reaction monitoring.

Table 4
%Conversion of MSA into EMS measured after 16 h under different reaction conditions.

Temperature	Water	Base	%Conversion after 16 h
40.2	No	No	0.009
50.0	No	No	0.036
60.0	No	No	0.123
69.7	No	No	0.350
69.7	No	No	0.325
69.9	No	No	0.342
69.9	5 vol.%	No	0.077
69.7	5 vol.%	No	0.075
40.3	5 vol.%	No	0.001
70.0	0.7%	Lutidine substoech[a]	0.004
70.0	0.7%	Lutidine excess[b]	<0.001
70.0	No	Hunig's[c]	<0.001

[a] 2% sub-stoechiometric to MSA.
[b] 10% excess to MSA.
[c] Hunig's base: disisopropylethylamine.

As an example, the results of a reaction monitoring are shown. From premixed MSA/ethanol reaction mixtures in tray C, maintained at 70 °C, 20 μL aliquots were removed every hour during 32 h (one vial per time point is used). The measured relative peak areas (formed EMS versus IS2) as a function of reaction time are shown in Fig. 4A and B. Fig. 4A shows the values initially obtained using a block-heated tray C with 32 positions for 10 mL vials. Although a clear trend is observed in this plot, some strange "drops" are observed. It became clear that the largest deviations are obtained for time points 8, 16, 24, etc. These time points correspond to the vial positions at the front side of the tray and measurement of the temperature inside these positions showed that the temperature was up to 4 °C lower than in other positions.

The tray was changed into a 98 position 2 mL vial tray which was heated using a thermostated water bath. The results of the same EMS monitoring in function of time performed using the new tray is displayed in Fig. 4B. Now the curve is much smoother enabling curve fitting and reaction kinetic studies. This tray type was used for further work.

To illustrate the applicability of the presented method, the formation of EMS from MSA and ethanol under different reaction temperatures is demonstrated in Fig. 5A. These reactions were performed in anhydrous conditions during reaction times up to 20 h. The important influence of temperature is clearly demonstrated. The influence of water is illustrated in Fig. 5B. The reaction of MSA in ethanol was monitored at 70 °C during 17 h. In anhydrous conditions, the conversion rate is approximately 0.35%. If water is present in the reaction mixture, this conversion yield rapidly drops.

As an illustration of the formation of EMS from MSA and ethanol, some values of conversion percentage after 16 h using different reaction mixtures are summarized in Table 4. First of all, it can be seen that conversion rates between 0% and 0.5% are observed, corresponding to the validated calibration range of the described method. In addition, the triplicate experiments at 70 °C (no water, no base) and duplicate experiments at 70 °C with 5% water (no base), show excellent repeatability in measured % conversion.

From this table it is also clear that the highest % conversion is obtained at the highest temperature, under anhydrous conditions without the presence of a base. At lower temperature and/or in the presence of water, the % conversion is drastically reduced. In the presence of bases, the acid is also neutralized (partially or complete), resulting in low formation yields. Interesting to note is that even a sub-stoichiometric amount of a weak base (2,6-lutidine), a situation reflecting API salt formation, shows a dramatic reduction of EMS formation (0.004%) versus the worse case (anhydrous, 70 °C).

Using the described method, the reaction mechanism and reaction kinetics controlling the formation of sulphonate esters from sulphonic acids and low molecular weight alcohols could be studied. The results of these studies are published elsewhere [12].

4. Conclusion

Using an automated derivatisation–static headspace-GC–MS method, the formation of ethyl methane sulfonate from methane sulphonic acid and ethanol can be monitored. The automated system allowed unattended operation over long time periods and provided excellent repeatability, linearity and robustness. The same system was applied for kinetic studies on the formation of EMS from MSA and ethanol under different reaction conditions. The procedure can and has been successfully applied to study the interaction of other alcohols (methanol and isopropanol) with methanesulfonic acid and alcohols with toluene sulfonic acid.

Acknowledgements

The authors thank the Product Quality Research Institute (PQRI) and the member companies for their financial support in realizing this project.

References

[1] A.T.M. Serajuddin, Adv. Drug. Deliv. Rev. 59 (2007) 603–616.
[2] S. Glowienke, W. Frieauff, Th. Allmendinger, H.-J. Martus, W. Sutter, L. Mueller, Mutat. Res. 581 (2005) 23–34.
[3] Guideline on the Limits of Genotoxic Impurities, Committee for Medicinal Products (CHMP), European Medicines Agency, London, 28 June 2006 (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006).
[4] T. McGovern, D. Jacobson-Kram, TrAC 25 (2006) 790–795.
[5] D.P. Elder, A. Teasdale, A.M. Lipczynski, J. Pharm. Biomed. Anal 46 (2008) 1–8.
[6] H.R.G. Ramijt, M.M. Singh, A.B. Coddington, J. Mass Spectrom 31 (1996) 867–872.
[7] W. Li, J. Chromatogr. A 1046 (2004) 297–872.
[8] G.E. Taylor, M. Gosling, A. Pearce, J. Chromatogr. A 1119 (2006) 231–237.
[9] I. Colon, S.M. Richoll, J. Pharm. Biomed. Anal 39 (2005) 477–485.
[10] C.R. Lee, F. Guivarch, C.N. Van Dau, D. Tessier, A.M. Krstulovic, Analyst 128 (2003) 857–863.
[11] R. Alzaga, R.W. Ryan, K. Taylor-Worth, A.M. Lipczynski, R. Szucs, P. Sandra, J. Pharm. Biomed. Anal 45 (2007) 472–479.
[12] A. Teasdale, S.C. Eyley, E. Delaney, K. Jacq, K. Taylor-Worth, A. Lipczynski, V.D. Reif, D.P. Elder, K.L. Facchine, S. Golec, R. Schulte Oestrich, P. Sandra, F. David, OPRD, (2008), in press.