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Gas chromatographic–mass spectrometry method for the detection of busulphan and its metabolites in plasma and urine

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ABSTRACT

Busulphan is an alkylating agent used as conditioning regimen prior to stem cell transplantation. Busulphan is metabolized in the liver and four major metabolites have been identified. The first metabolite is tetrahydrothiophene which is oxidized to tetrahydrothiophene 1-oxide, then sulfolane and finally 3-hydroxy sulfolane. Despite the low molecular weight and wide polarity range of busulphan and its four metabolites, the use of a fused silica non-polar column significantly enhanced the automated gas chromatography–mass spectrometry of their detection in one simple method. The limit of quantification was 0.5 μM for busulphan and all its metabolites except 3-OH sulfolane, which was 1.25 μM. This method was validated for all the compounds in both human plasma and urine. Lower limits of quantifications (LLOQs) were run in pentaplicate per compound and all results were within 20% of the nominal values. The recovery was determined by comparing the peak area of two quality control (QC) samples, before and after extraction in plasma and urine, in triplicate. Acceptable precision and accuracy have been obtained; at least 3 standard curves have been run for each compound using three different QCs covering the calibration curve in triplicate. The QC values were within 15% (SD) of the nominal values. Selectivity and sensitivity of all compounds have been measured. Compounds were stable up to 50 days after extraction in −20 °C and 48 h at RT. Moreover, the compounds were stable for three cycles of freezing and thawing. The method was applied in a clinical case where the patient received high dose busulphan; all the compounds have been detected, identified and quantified both in plasma and urine.

1. Introduction

Mass spectrometry (MS) is a selective analytical method that measures the mass-to-charge ratio of charged particles. The use of a mass spectrometer as a detector in gas chromatography was developed during the 1950s [1].

Gas chromatography–mass spectrometry (GC–MS) is still the method of choice where thermo stable and low molecular weight compounds are concerned. Applications of GC–MS include environmental monitoring and cleanup, law enforcement, security, astrochemistry and drug detection. It also plays a major role in forensic medicine.

Busulphan (Bu) is an alkylating agent used in high doses as conditioning regimen prior to stem cell transplantation (SCT). It acts by producing guanine–adenine intrastrand cross linking in the DNA that trigger apoptosis and cell death.

Busulphan is predominantly metabolized in the liver through an enzymatic conjugation with glutathione (GSH) (Fig. 1) [2–5]. Four metabolites have been detected and identified in rat and human plasma and urine and several minor metabolites of Bu have been detected but yet not identified [2–5].

The conjugation of busulphan with glutathione results in a sulfonium ion which is positively charged. This sulfonium ion is an intermediate unstable product and can be cleaved to tetrahydrothiophene (THT) either enzymatically or chemically [6]. THT is a lipophilic compound that exists as a metabolite of several xenobiotics [7]. THT is normally reabsorbed and oxidized to tetrahydrothiophene 1-oxide (THT 1-oxide) [3]. THT 1-oxide is further oxidized to sulfolane (tetrahydrothiophene 1,1-dioxide), then to 3-hydroxysulfolane (3-OH sulfolane).

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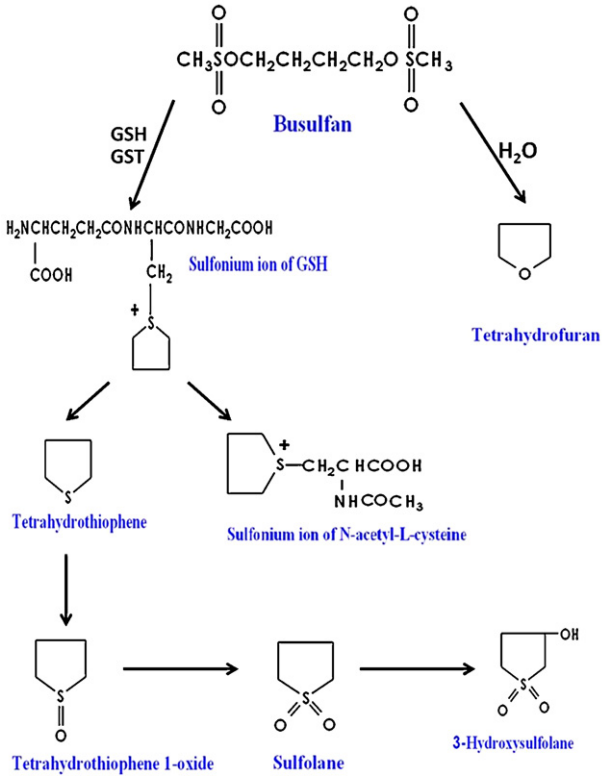

Fig. 1. Metabolic pathway of busulphan.

Dibromoalkanes belong to the lipophilic and electrophilic xenobiotics. 1,4-dibromobutane has a structural similarity to busulphan in its metabolism and forms cyclic sulfonium ions which decompose to THT [7].

THT is an organosulfur compound that recently has been proved to be an important element in the synthesis and structures of cyclic gold complexes [8,9].

THT 1-oxide is a non-toxic metabolite of busulphan and is also important in the field of bacteriology. Zinder et al. reported that THT 1-oxide stimulates the growth of an unidentified bacterium [10]. Later, it was found that THT 1-oxide is important for the anaerobic growth of *Escherichia coli* [11].

Sulfolane is known as a water miscible chemical used in the sweetening of sour gas. It is used to remove toxic levels from sour gases like hydrogen sulphide from natural gas condensate in combination with diisopropanolamine [12]. Sulfolane is usually investigated in agriculture from wetland vegetation like roots, seeds, leaves and even from the soil to determine the extent of translocation within plants [13]. Sulfolane is also one of the recalcitrant organic compounds (ROCs) that are utilized in industrial processes and considered xenobiotic. Some bacterial species are able to metabolize sulfolane from water slowly [14,15].

3-OH sulfolane is considered the final metabolite of busulphan by adding a hydroxyl group to sulfolane [3]. Another study of dibromoalkanes reported that 3-OH sulfolane is formed after oxidation of THT [7]. 3-OH sulfolane is also involved in HIV treatment as it is known that HIV protease is vital for propagation, and the inhibition of this protease is one of the therapeutic targets for AIDS treatment [16–18].

In the present investigation we utilized GC–MS as one simple and reproducible method for the detection of busulphan and its metabolites despite their different methods of extraction. We have examined several columns for these analyses; however, the 100% dimethyl polysiloxane fused silica non-polar phase columns were the most suitable to separate all the compounds although they have low molecular weight and different polarity. These columns showed high accuracy, reproducibility and stable retention time.

In the present investigation we utilized SIM mode in the detection due to high interference from the endogenous compound when analyzing plasma and urine, especially after extraction by lyophilization.

The detection of busulphan and its metabolites in patients is important to understanding the metabolic pathway, toxicity and kinetics of the drug, and hence obtaining better treatment efficacy.

2. Experimental

2.1. Chemicals and reagents

The following compounds were purchased with a purity (>96%): busulphan 17- and 3-hydroxysulfolane (Sigma–Aldrich, Steinheim, Germany), tetrahydrothiophene and sulfolane (Sigma–Aldrich, St. Louis, USA) and tetrahydrothiophene 1-oxide (Sigma–Aldrich, Tokyo, Japan) sodium iodide (Merck, Darmstadt, Germany), nicotine (Merck, Hoherbrunn, Germany) and 3-methylsulfolane (TCI, Tokyo, Japan). 1,5-Bis(methanesulfonoxy)pentane was prepared in our laboratory (Karolinska University Hospital-Huddinge, Sweden) [19].

All solvents were of analytical grade with purity (>99%): methanol, acetonitrile, n-heptane (Merck, Darmstadt, Germany), dichloromethane (Fluka, Seeze, Germany) and ethyl acetate (Sigma–Aldrich, Steinheim, Germany).

Heparinized pooled plasma from healthy individuals, obtained from the blood and transfusion center at The Karolinska University Hospital-Huddinge, was stored at −20 °C until used. Urine from healthy individuals was freshly collected, pooled and used.

2.2. Instrument

The GC–MS system consisted of a Hewlett Packard 5890 Series II gas chromatograph equipped with an Agilent 6890 series autoinjector and Hewlett Packard 5972 series mass selective detector (Santa Clara, CA, USA). The column used was Rxi-1ms 100% dimethyl polysiloxane split fused silica non-polar phase, 30 m, 0.25 mm ID and 1.0 μm df (Bellefonte, PA, USA). The carrier gas was helium and the pressure on the top of the column was 40 kPa with an inlet temperature of 50 °C. A 2 μL sample aliquot was injected splitless at a flow of 0.9 mL/min. The analysis was run in a gradient with an initial temperature of 40 °C for 2.5 min followed by 20 °C min^{-1} increase up to 100 °C then 10 °C min^{-1} increase up to 180 °C and finally 50 °C min^{-1} increase up to 240 °C. The final temperature was maintained for 3 min. The injector temperature was 190 °C while the detector temperature was 250 °C [6].

Mass spectrometry was operated in selective ion monitoring (SIM) at the following ions: *m*/*z* 310 for busulphan after conversion to 1,4-diiodobutane, *m*/*z* 60 and 88 for THT, *m*/*z* 55 and 104 for THT 1-oxide, *m*/*z* 56 and 120 for sulfolane and *m*/*z* 44 and 136 for 3-OH sulfolane. For compounds used as internal standard, *m*/*z* 324 for 1,5-bis(methanesulfonoxy)pentane after conversion to 1,5-diiodopentane, *m*/*z* 162 for nicotine and *m*/*z* 134 for 3-methylsulfolane. These are the parent compounds and the highest daughter ions detected.

2.3. Sample preparation

Frozen plasma samples were thawed at room temperature and vortexed before use, while urine samples were freshly collected at the same day. The analysis was performed for each analyte separately (3 injections) and was also evaluated after mixing the analytes and injecting once. All stock solutions were prepared as 1 mM solutions (THT, THT 1-oxide, sulfolane and 3-OH sulfolane

were dissolved in phosphate buffer pH (7.4) when added to plasma or urine, while busulphan was dissolved in acetonitrile), serial dilutions were prepared from the stock to the appropriate concentrations. For the recovery study, all compounds were prepared as stock solutions (1 mM) in the appropriate solvent and subsequently diluted in the same solvent in serial dilutions prior to use.

2.3.1. Busulphan

Busulphan (50 μL) was added to plasma or urine (450 μL). The matrix was then extracted according to the method reported previously by Hassan et al. [20]. Briefly, 500 μL of plasma and urine were diluted with 500 μL of H_2O. Fifty microliter of the internal standard (1,5-bis(methanesulfonoxy)pentane; 38.5 μM) were added, followed by the addition of 1 mL of sodium iodide (8 M) and 400 μL of n-heptane. The tubes were placed, under magnetic stirring, in a water bath (70 °C) for 45 min. During the reaction, busulphan and 1,5-bis(methanesulfonoxy)pentane were converted to 1,4-diiodobutane and 1,5-diiodopentane respectively and extracted to n-heptane [19]. The organic phase was transferred into GC–MS tubes. 1,4-Diiodobutane and 1,5-diiodopentane were detected at SIM mode with the ions *m*/*z* 310 and *m*/*z* 324 at retention times of 14.04 and 15.12 min respectively (Supplementary, Fig. 1). The percentage of the conversion of busulphan and 1,5-bis(methanesulfonoxy)pentane to 1,4-diiodobutane and 1,5-diiodopentane was over 90% [20].

2.3.2. Tetrahydrothiophene and sulfolane

THT and sulfolane (20 μL) were added to plasma or urine (160 μL), nicotine 10 μM (20 μL) was added before extraction as an internal standard. The matrix was extracted by liquid–liquid extraction with dichloromethane (equal volumes 200 μL) for 30 s of high speed vortex. After extraction and centrifugation for 10 min (16,000 × *g*), the organic phase was transferred into GC–MS tubes. THT and sulfolane were detected at SIM mode with the ions *m*/*z* 60 and *m*/*z* 56 at retention times of 7.17 and 12.57 min respectively and *m*/*z* 162 for nicotine at 14.79 min (Supplementary, Fig. 2).

2.3.3. Tetrahydrothiophene 1-oxide and 3-hydroxysulfolane

THT 1-oxide and 3-OH sulfolane (20 μL) were added to plasma or urine (160 μL). 10 μM of 3-methylsulfolane (20 μL) was added as an internal standard. The matrix was lyophilized (<1 mbar at 40 °C under N_2 stream) to dryness; the residue was dissolved in 10 μL water and an equal volume of ethyl acetate (200 μL) for extraction. Samples were vortexed for 30 s at high speed and the organic phase was transferred to GC–MS tubes. THT 1-oxide and 3-OH sulfolane were detected at SIM mode with the ions *m*/*z* 55 and *m*/*z* 44 at retention times of 11.63 and 13.19 min respectively, while it was *m*/*z* 134 for 3-methylsulfolane at 15.06 min (Supplementary, Fig. 3).

2.4. Conduct of validation

The bioanalytical validation has been done according to the international guidelines recommended by Shah et al. [21,22]. A standard curve was defined by a set of calibration standards ranging between 0.5 μM and 50 μM for all compounds except 3-OH sulfolane, which was between 1.25 μM and 50 μM, randomized through the entire run. At least three standard curves per analyte were assayed over a 3-day period in both plasma and urine. The quantification analysis was based on the ratio peak area analyte/peak area IS and equal weighting in a linear regression analysis equation. Three quality controls (QCs) covering the whole range of the standard curve (2, 15 and 30 μM) have been run in triplicate over 3 days in both plasma and urine to determine the inter- and intra-day reproducibility. Each run also included blank samples for all compounds and were compared with the lower limits of quantifications (LLOQs). Stability of the analytes in biological fluids and through the analytic process was established with two different concentrations of QC [21,22].

2.5. Clinical application

In order to perform dose adjustment for busulphan, blood samples were withdrawn routinely from a patient undergoing stem cell transplantation. The patient was treated with high dose busulphan (2 mg/kg bid) for 4 days. Samples were collected at 0, 0.5, 1,2,3, 4, 6, 8, and 10 h for the first and fifth dose and at 0, 1, 3 and 6 h for doses 3 and 8. Blood was collected in EDTA vacutainer tubes, plasma was spearated by centrifugation at 1200 × *g* and stored at −20 °C until analysis as described above. Urine samples were collected at 6, 12, 24, 48 and 72 h and stored at −20 °C until assayed.

2.6. Analytic data treatment

Chromatograms and all the quantitative results were measured using Enhanced ChemStation software G1401BA version B.01.00 (Agilent, Santa Clara, CA, USA) while the statistical results were obtained using both Prism ver. 4 (Graph Pad, CA, USA).

3. Results and discussion

3.1. Choice of a solvent

Extraction solution could significantly affect the amount of extract, peak shape and column efficiency [23]. Different solutions have been tried with all the compounds to reach the maximum recovery, highest sensitivity for the compound analyzed expressed as the lowest LLOQ and best peak shape. Solvents that have been tested included hexane, pentane, toluene and diethyl ether. Our trials resulted in the three solvents defined previously with three methods of extraction for the five compounds to reach the best results.

3.2. Quantitative analysis of the analytes in biological fluids

3.2.1. Sensitivity and selectivity

LLOQ chromatograms of busulphan and its internal standard after conversion to 1,4 diiodo-butane and 1,5 diiodo-pentane compared to the blank plasma are shown in Fig. 2, while Fig. 3 shows LLOQ of THT, sulfolane and blank plasma. Fig. 4 shows LLOQ of THT 1 oxide and 3 hydroxysulfolane. Matrix effect bias of both plasma and urine has been discussed as a major problem for quantification with GC–MS, but using SIM mode reduced this effect. Five samples of each compound have been run over 3 days in both plasma and urine. The LLOQ was 0.5 μM in all compounds except 3-OH sulfolane which was 1.25 μM. Blank samples have been run parallel to the LLOQ samples and no carry over was observed. This is probably due to the extensive washing procedure between samples (5× solvent and 5× acetone). Results for LLOQ are shown in Table 1. All the results were within 20% of the expected value.

3.2.2. Recovery

The recovery was determined by comparing the concentrations of two QC samples after extraction from plasma and urine with the same concentration dissolved in solvent and injected directly to GC–MS. QC samples have been run in triplicate and results are shown in Table 2. Sulfolane showed the best recovery (93.2%) while THT had the lowest recovery value (17.1%). The recoveries of THT 1-oxide and 3-hydroxy sulfolane were 44.4% and 48.2%, respectively, while for the internal standards nicotine and 3-methylsulfolane the recoveries were 93.5% and 70%,

Table 1
LLOQ in biological fluids (n = 15 per analyte); SD, standard deviation.

	Plasma			Urine		
	Mean	SD	RSD%	Mean	SD	RSD%
Busulphan 0.5 μM	0.483	0.087	18.01	0.585	0.114	19.49
Tetrahydrothiophene 0.5 μM	0.469	0.064	13.65	0.510	0.069	13.53
Tetrahydrothiophene 1-oxide 0.5 μM	0.567	0.094	16.58	0.545	0.080	14.68
Sulfolane 0.5 μM	0.568	0.106	18.66	0.422	0.080	18.96
3-Hydroxysulfolane 1.25 μM	1.413	0.074	5.24	1.071	0.138	12.89

Table 2
Recovery from biological fluids after extraction (n = 9–12 per analyte); SD, standard deviation.

	Mean%	SD%
Busulphan	91	3.2
Tetrahydrothiophene	17.1	8.1
Tetrahydrothiophene 1-oxide	44.4	1.5
Sulfolane	93.2	12.2
3-Hydroxysulfolane	48.2	4.2
1,5-Bis(methanesulfonoxy)pentane	92	3.5
Nicotine	93.5	2.0
3-Methylsufolane	70.0	6.9

respectively. The recoveries of busulphan and its internal standard (1,5-bis(methanesulfonoxy)pentane) were 91% and 92%, respectively, which is in good agreement with the results reported previously [20].

3.2.3. Accuracy and precision

Accuracy and precision were established from the analysis of three standard curves and three QCs in triplicates using both biological fluids (Supplementary Table 1). The standard curve was lineal over the concentrations range of 0.5–50 μM for busulphan, THT, THT 1-oxide and sulfolane and 1.25–50 μM for 3-OH sulfolane. The standard curves were run for each compound and all calibration curves contained between 5 and 7 standard points. R^2 for all curves is between 0.995 and 0.999.

The precision and accuracy were determined for all five analytes in triplicate for inter- and intra-day variations over three consecutive days. The QC results showed a standard deviation <15% for all values obtained including low, medium and high QCs compared to the nominal values (Table 3).

Calibration standards and QCs were prepared fresh daily by dilution of individual aliquots of stock solution(s). The final percentage of the biological matrix in the quality control samples was at least 80%.

3.2.4. Sample stability

Stability tests of the analytes in the sample extract were established. Two quality controls have been run in triplicate after extraction from both plasma and urine. Results are listed in supplementary section (Tables 2 and 3). The analytes were stable during the extraction process, extraction storage and chromatography. Since all the compounds are not known to be light sensitive; they have been stored on a bench in daylight.

The stability of the analytes was also examined prior to extraction in plasma and urine (Supplementary Table 4). The results showed that all compounds were stable at 4 and −20 °C for 24 h. However, at RT for 24 h, the concentration of THT was decreased by about 38% in urine while it was more stable in plasma, where the decrease was only 17%; busulphan concentrations decreased by about 34% in plasma and were stable in urine. THT 1-oxide, sulfolane and 3-OH sulfolane were stable in both plasma and urine at

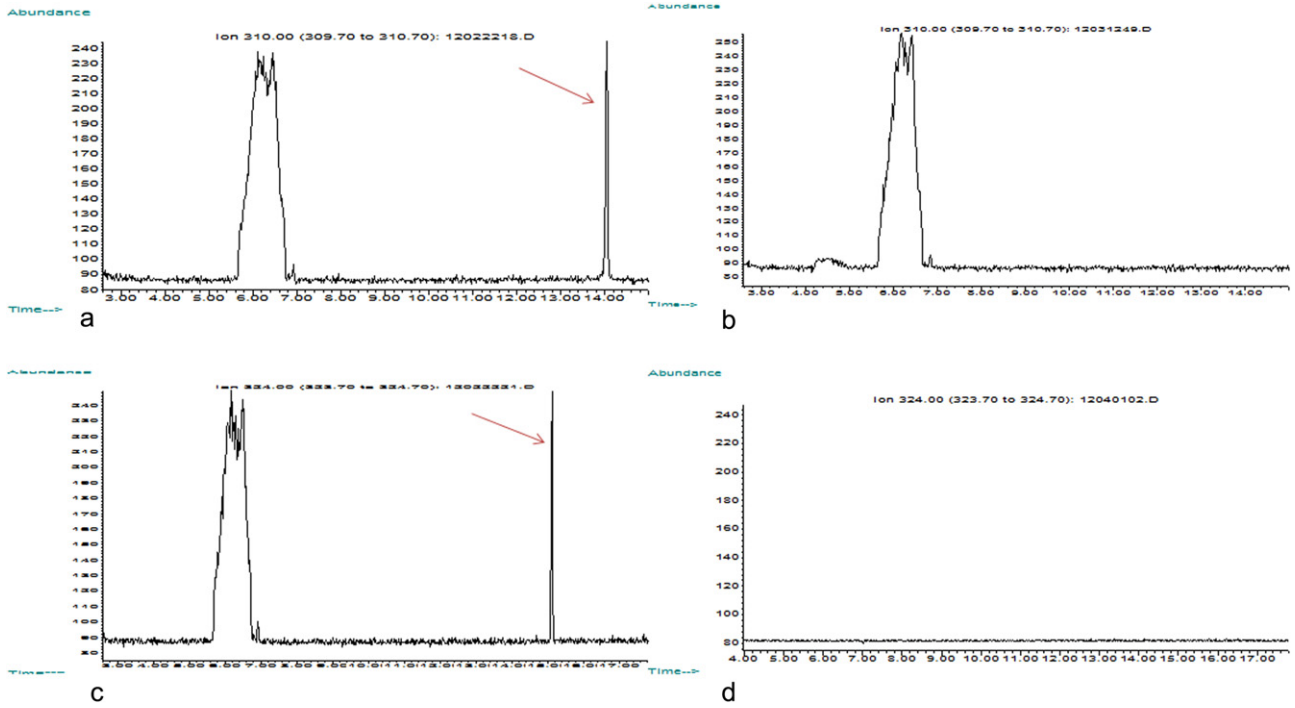

Fig. 2. GC–MS on SIM mode for (a) LLOQ for busulphan after extraction and conversion to 1,4-diiodobutane compared with (b) blank for busulfan and (c) 1,5-bis(methanesulfonoxy)pentane as an internal standard after extraction and conversion to 1,5-diiodopentane compared with (d) blank for 1,5-bis(methanesulfonoxy)pentane.

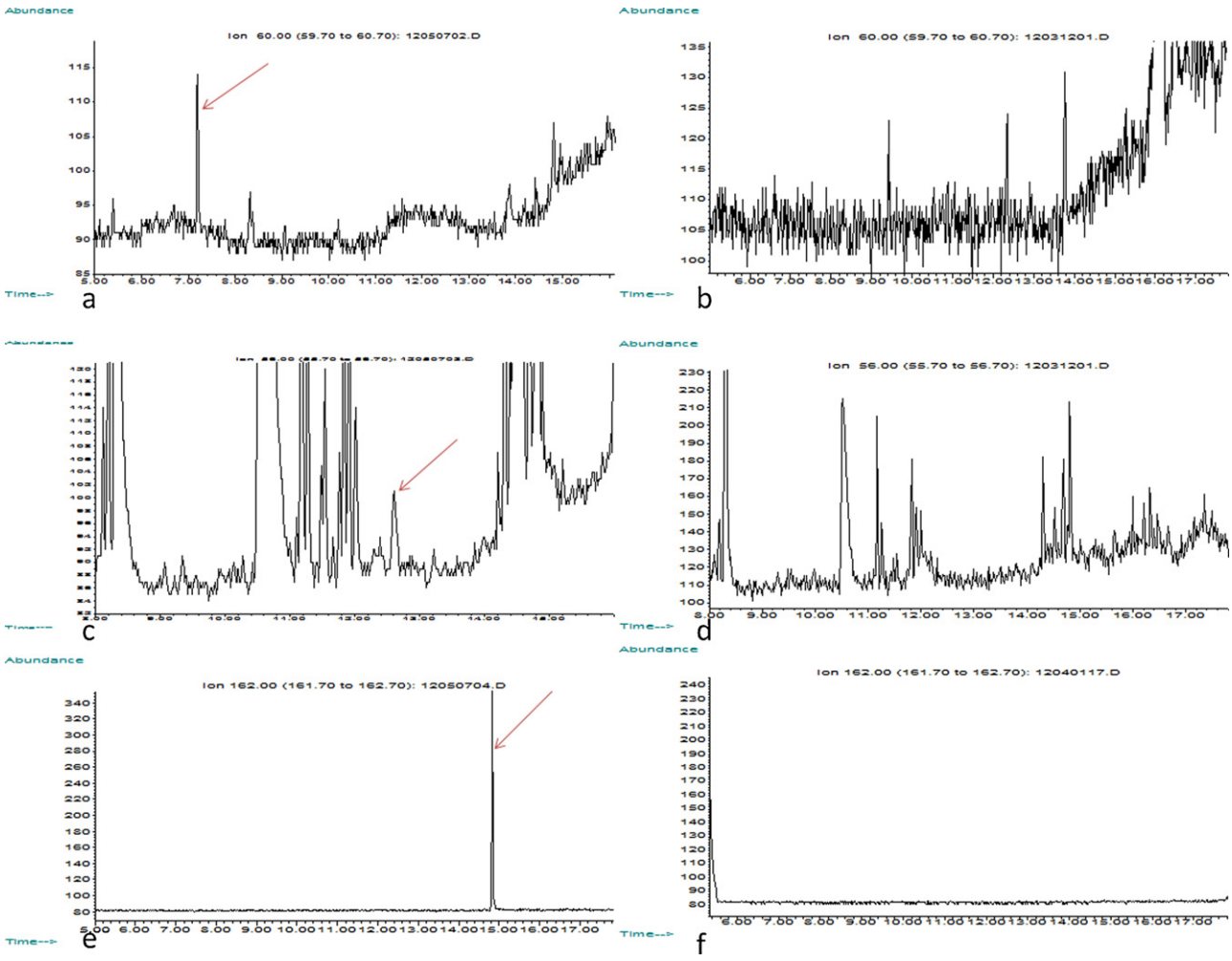

Fig. 3. GC–MS on SIM mode for (a) LLOQ for THT after extraction compared with (b) blank for THT and (c) LLOQ for sulfolane after extraction compared with (d) blank for sulfolane and (e) nicotine as an internal standard for both of them compared with (f) blank for nicotine.

RT for 24 h. Our results indicate that samples contacting THT and busulphan should be stored immediately at 4 or −20 °C, while for the other analytes RT can be sufficient for 24 h.

3.2.5. Clinical application

Fig. 5 shows busulphan concentrations in patient plasma with complete sampling protocol for the first and third doses. First AUC was calculated to 7240 ng/mL min; by adjusting the dose to reach the target AUC, the AUC was calculated to 8151 and 11604 for doses 5 and 8. The levels of THT were rather low indicating rapid metabolism (oxidation). A continuous increase in both THT 1-oxide and higher levels of sulfolane indicates further oxidation steps, while the low levels of 3-OH sulfolane indicate the elimination of the metabolite through the kidneys as can be seen in Fig. 6.

Table 3
Quality controls in biological fluids ($n = 9$ per analyte); SD, standard deviation.

		Plasma			Urine		
		Mean	SD	RSD%	Mean	SD	RSD%
Busulphan	Low 2 μM	1.875	0.243	12.960	2.267	0.234	10.322
	Medium 15 μM	17.20	1.613	9.378	13.456	0.40	2.973
	High 30 μM	32.267	1.956	6.062	30.444	1.862	6.116
Tetrahydrothiophene	Low 2 μM	1.778	0.188	10.574	2.166	0.11	5.078
	Medium 15 μM	14.144	1.368	9.672	12.814	0.858	6.696
	High 30 μM	30.2	2.506	8.298	25.5	3.359	13.173
Tetrahydrothiophene 1-oxide	Low 2 μM	2.133	0.292	13.690	1.867	0.166	8.891
	Medium 15 μM	15.372	1.742	11.332	14.516	1.292	8.901
	High 30 μM	31.878	3.026	9.492	30.211	1.518	5.025
Sulfolane	Low 2 μM	1.831	0.073	3.987	1.879	0.248	13.199
	Medium 15 μM	15.056	1.102	7.319	13.678	1.445	10.564
	High 30 μM	30.944	1.053	3.403	28.111	3.195	11.366
3-Hydroxysulfolane	Low 2 μM	2.14	0.286	13.364	2.244	0.270	12.032
	Medium 15 μM	17.144	1.579	9.210	13.625	1.807	13.262
	High 30 μM	33.333	3.228	9.684	29.556	1.884	6.374

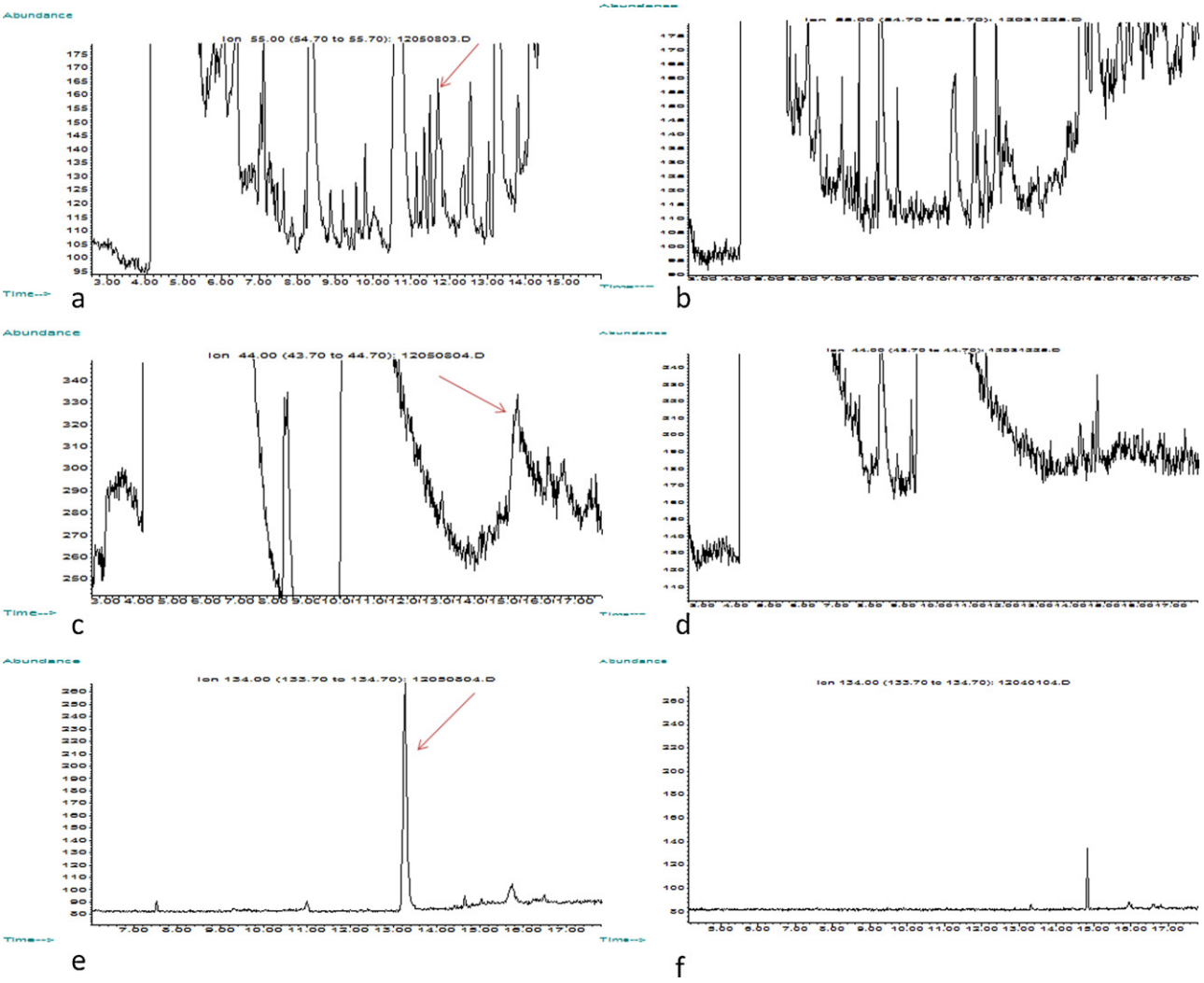

Fig. 4. GC–MS on SIM mode for (a) LLOQ for 1 oxide THT after extraction compared with (b) blank for 1 oxide THT and (c) LLOQ for 3-hydroxysulfolane after extraction compared with (d) blank for 3-hydroxysulfolane and (e) 3-methylsulfolane as an internal standard for both of them compared with (f) blank for 3-methylsulfolane.

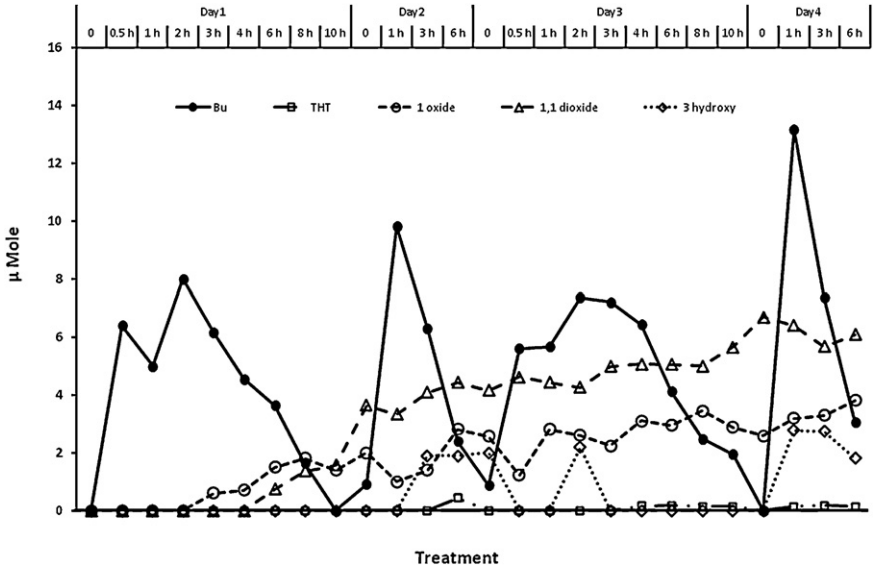

Fig. 5. Busulphan and its metabolites in patient plasma during 4 days of high dose treatment.

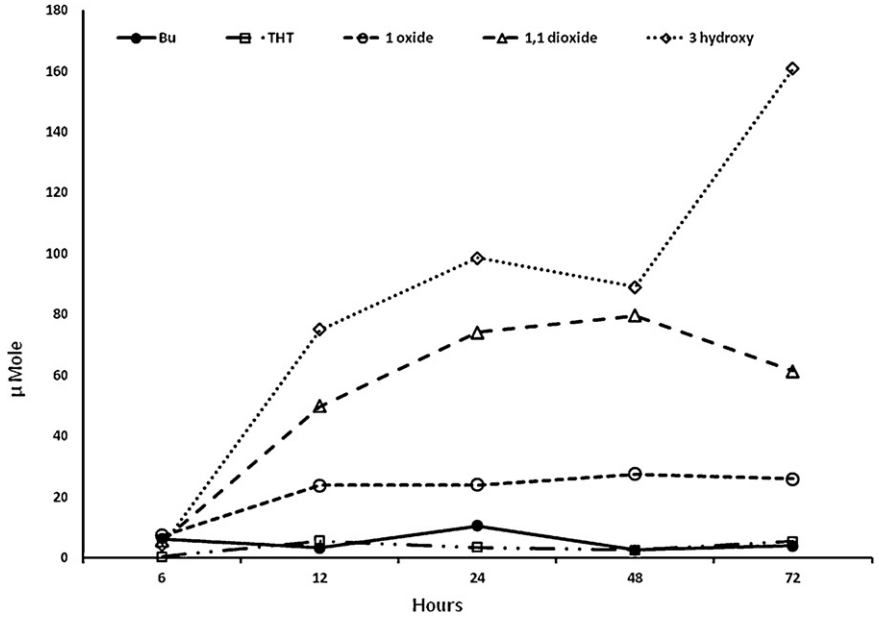

Fig. 6. Busulphan and its metabolites in patient urine during 4 days of high dose treatment.

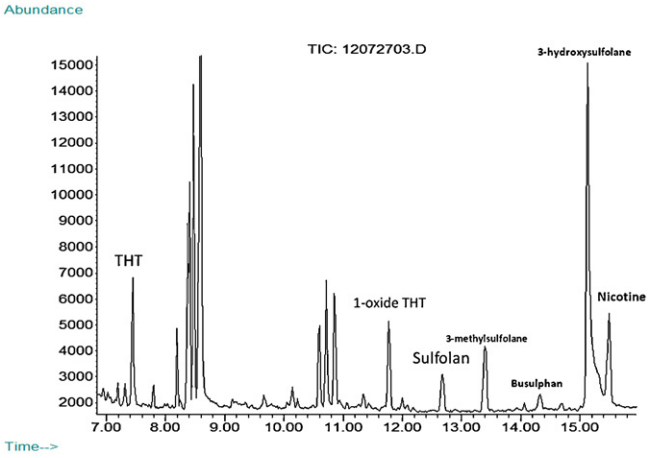

Fig. 7. GC–MS chromatogram showing busulphan, THT, THT 1-oxide, sulfolane, 3-hydroxy sulfolane, nicotine and 3-methyl sulfolane using TIC mode.

Moreover, it can also be observed that the highest concentration of the metabolites after 4 days treatment is 3-OH sulfolane (Fig. 6), which is the most polar compound followed by sulfolane and to a lesser extent THT 1-oxide. The levels of busulphan and THT were very low due to their lipophilicity. These results show that 3-OH sulfolane is the last oxidized metabolite which is excreted into the urine due to its hydrophilic nature.

4. Conclusion

We have developed a new, reproducible and robust method for the detection of busulphan and four of its major metabolites in both plasma and urine in one analysis, regardless of different methods of extraction (Fig. 7). Despite the difference in molecular weight and polarity of busulphan and its four metabolites, fused silica non-polar column in combination with GC–MS resulted in an accurate analysis of all the compounds and provided reproducible results. The detection of Bu and its metabolites in patients is important for understanding the metabolic pathway and toxicity of busulphan, as well as the pharmacokinetics and pharmacodynamics of the drug. Optimizing high dose chemotherapy will certainly reduce the adverse effects and enhance clinical outcome.

Moreover, some of these metabolites are used in other non-medical fields and are vital for agriculture and environmental medicine. Use of the present developed method may enhance extraction and detection of these compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.12.001.

References

[1] R.S. Gohlke, F.W. Mclafferty, J. Am. Soc. Mass Spectrom. 4 (1993) 367.
[2] M. Hassan, H. Ehrsson, Eur. J. Drug Metab. Pharmacokinet. 12 (1987) 71.
[3] M. Hassan, H. Ehrsson, Drug Metab. Dispos. 15 (1987) 399.
[4] M. Hassan, H. Ehrsson, I. Wallin, S. Eksborg, Eur. J. Drug Metab. Pharmacokinet. 13 (1988) 301.
[5] M. Hassan, G. Oberg, H. Ehrsson, M. Ehrnebo, I. Wallin, B. Smedmyr, T. Totterman, S. Eksborg, B. Simonsson, Eur. J. Clin. Pharmacol. 36 (1989) 525.
[6] C.A. Ritter, F. Bohnenstengel, U. Hofmann, H.K. Kroemer, B. Sperker, J. Chromatogr. B: Biomed. Sci. Appl. 730 (1999) 25.
[7] W. Onkenhout, W.M. van Loon, W. Buijs, A. van der Gen, N.P. Vermeulen, Drug Metab. Dispos. 14 (1986) 608.
[8] S. Bhargava, K. Kitadai, T. Masashi, D.W. Drumm, S.P. Russo, V.W. Yam, T.K. Lee, J. Wagler, N. Mirzadeh, Dalton Trans. 41 (2012) 4789.
[9] J.A. Cabeza, J.M. Fernandez-Colinas, P. Garcia-Alvarez, D. Polo, Inorg. Chem. 51 (2012) 3896.
[10] S.H. Zinder, T.D. Brock, Arch. Microbiol. 116 (1978) 35.
[11] R. Meganathan, J. Schrementi, J. Bacteriol. 169 (1987) 2862.
[12] J.V. Headley, K.M. Peru, L.C. Dickson, J. Chromatogr. A 859 (1999) 69.
[13] R. Saint-Fort, J. Environ. Sci. Health A: Tox. Hazard. Subst. Environ. Eng. 41 (2006) 1211.

[14] W.J. Lee, J.K. Lee, J. Chung, Y.J. Cho, D.H. Park, J. Microbiol. Biotechnol. 20 (2010) 1230.
[15] E.A. Greene, P.H. Beatty, P.M. Fedorak, Arch. Microbiol. 174 (2000) 111.
[16] A.K. Ghosh, W.J. Thompson, H.Y. Lee, S.P. McKee, P.M. Munson, T.T. Duong, P.L. Darke, J.A. Zugay, E.A. Emini, W.A. Schleif, et al., J. Med. Chem. 36 (1993) 924.
[17] A.K. Ghosh, H.Y. Lee, W.J. Thompson, C. Culberson, M.K. Holloway, S.P. McKee, P.M. Munson, T.T. Duong, A.M. Smith, P.L. Darke, et al., J. Med. Chem. 37 (1994) 1177.
[18] S.P. Gupta, M.S. Babu, S. Sowmya, Bioorg. Med. Chem. 6 (1998) 2185.
[19] H. Ehrsson, M. Hassan, M. Ehrnebo, M. Beran, Clin. Pharmacol. Ther. 34 (1983) 86.
[20] M. Hassan, H. Ehrsson, J. Chromatogr. 277 (1983) 374.
[21] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, et al., Eur. J. Drug Metab. Pharmacokinet. 16 (1991) 249.
[22] V.P. Shah, K.K. Midha, J.W. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551.
[23] W. Naidong, W. Shou, Y.L. Chen, X. Jiang, J. Chromatogr. B: Biomed. Sci. Appl. 754 (2001) 387.