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Development and validation of an LC–MS/MS method for determination of methanesulfonamide in human urine

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ABSTRACT

A sensitive and selective liquid chromatographic method coupled with tandem mass spectrometry (LC–MS/MS) was developed and validated for the quantification of methanesulfonamide (MSA) in human urine. MSA is a potential in vivo metabolite of reparixin, a specific inhibitor of the CXCL8 biological activity. In this study, a simple derivatization procedure with a new reagent, *N*-(4-methanesulfonyl-benzoyl)-imidazole, was set up to enable MSA and the internal standard (I.S.), ethanesulfonamide (ESA), to be analysed by LC–MS/MS. After derivatization, samples were evaporated and reconstituted in 30% acetonitrile, aq. MSA and I.S. derivatives were separated by reversed phased HPLC (high performance liquid chromatography) on a Luna 5 μ C18 column and quantitated by MS/MS using electrospray ionization (ESI) and multiple reaction monitoring (MR M) in the negative ion mode. The most intense $[M-H]^-$ MRM transition of derivatized MSA at *m/z* 276.2 → 197.2 was used for quantitation and the transition at *m/z* 290.2 → 211.2 was used to monitor derivatized ESA. The method was linear over the concentration range from 1 to 100 μg/ml, with a lower limit of quantitation of 1 μg/ml. The intra- and inter-day precisions were less than 5.5% and 10.1%, respectively, and the accuracies were between −4.0% and +11.3%. The method was successfully applied to quantify levels of MSA in human urine after intravenous administration of reparixin to healthy volunteers.

1. Introduction

Acyl sulfonamides are widely employed in medicinal chemistry mainly as bioisosters of carboxylic acids. Several classes of acyl sulfonamides are under pharmacological or clinical characterization as prostaglandin fertility regulators as well as inhibitors of steroid sulphatase, HCV (hepatitis C virus) polymerase and carbonic anhydrase. Acyl methanesulfonamides of phenylpropionic acids have been characterized as CXCL8 inhibitors. Methanesulfonamide (Fig. 1A) is an expected metabolite of acyl sulfonamides, originated by hydrolysis of the amide bond of the parent molecule. Reparixin (formerly repertaxin), R-4-isobutyl-α-methylphenylacetyl-methanesulfonamide (Fig. 1B), is a specific inhibitor of CXCL8 biological activity, stemming from a Dompé drug design program for molecules intended to modulate chemokine action [1,2]. *In vitro* chemotaxis experiments have shown that reparixin inhibits human PMN (polymorphonuclear) migration induced by CXCL8 in the low nanomolar range. *In vivo*, reparixin prevented PMN infiltration into the reperfused transplanted kidney and kidney damage in rat models of delayed graft function (DGF). Moreover, reparixin prevented PMN infiltration and tissue damage in animal models of ischemia/reperfusion (I/R) injury of liver, brain, intestine, heart and spinal cord. Reparixin was also investigated in a rat model of lung transplantation where it produced a dramatic improvement in isolated graft oxygenation, reduced pulmonary oedema, and significantly reduced neutrophil infiltration into transplanted lung grafts with induced I/R injury. Reparixin is under phase 2 clinical development in USA, Canada and Europe for the prevention of delayed graft function (DGF)/primary graft disfunction (PGD) in kidney and lung transplantation.

For a better understanding of reparixin metabolism, a sensitive and precise analytical method is essential to determine the methanesulfonamide concentration in urine. However, there are no methods described in literature for the determination of MSA in human urine, and in a previous study the quantitative deter-

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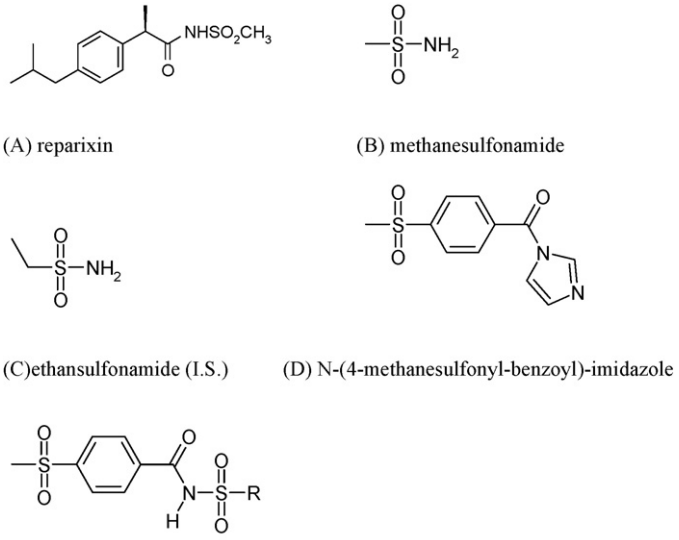

Fig. 1. (A–F): chemical structures of reparixin (A), methanesulfonamide (B), ethansulfonamide (C), *N*-(4-methanesulfonyl-benzoyl)-imidazole (D), *N*-(4-methanesulfonyl-benzoyl)-methanesulfonamide (E) and *N*-(4-methanesulfonyl-benzoyl)-ethanesulfonamide (F).

mination of MSA in cynomolgus monkey urine was performed by thin-layer chromatography and visualisation of radio-labelled material (carbon-14) by autoradiography [3].

It is difficult to detect MSA in biological fluids by standard chromatographic techniques such as either RP-HPLC (reversed phase-high performance liquid chromatography) due to its high polarity, to the lack of chromophores affecting UV (ultraviolet) detection and to its poor ionization capability affecting MS detection, or by capillary GC (gas chromatography), due to the very low volatility, to the poor sensitivity to FID (flame ionization detection) detection and to the low molecular weight (affecting MS detection) of this analyte.

This study was undertaken with the aim to develop a reliable and sensitive method for determination of MSA in human urine. This was accomplished by a novel derivatization procedure using *N*-(4-methanesulfonyl-benzoyl)-imidazole (Fig. 1D) to enable MSA and the internal standard ethanesulfonamide (ESA; Fig. 1C) to be analysed by LC–MS/MS. Detection of MSA and ESA derivatives was accomplished by liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI/MS/MS). The method was fully validated and used to assay MSA in human urine obtained from healthy volunteer treated intravenously with reparixin.

2. Experimental

2.1. Chemicals

MSA, ESA, ammonium formate and 1,8-Diazobicyclo[5-4-0]undec-7-ene (DBU) were purchased from Sigma–Aldrich Company, Poole, Dorset, UK. HPLC grade acetonitrile and glacial acetic acid were purchased from Fisher Scientific, Loughborough, Leicestershire, UK. HPLC grade water was obtained by Medeval Ltd., Manchester, UK. Derivatizing reagent *N*-(4-methanesulfonyl-benzoyl)-imidazole (Fig. 1D) was synthesized in-house, as detailed in the following section. Blank (analyte-free) human urine was collected by Medeval Ltd., Manchester, UK.

2.2. Synthesis of N-(4-methanesulfonyl-benzoyl)-imidazole

1,1′-Carbonyl-diimidazole (0.490 g, 3.0 mmol) was added to a suspension of 4-(methanesufonyl)-benzoic acid (0.60 g, 3.0 mmol) in dried THF (tetrahydrofuran) (7 ml). After a few minutes the solution was completely clear. The mixture was stirred at room temperature for 3 h. The obtained precipitate was filtered off and washed with THF dry to give pure *N*-(4-methansulfonyl-benzoyl)-imidazole (0.675 g, 2.7 mmol) as white solid, yield 90%. ^{1}H NMR ($CDCl_3$) δ 8.18 (d, 2H, J = 8 Hz), 8.08 (s, 1H), 8.00 (d, 2H, J = 8 Hz), 7.52 (d, 1H, J = 1 Hz), 7.22 (d, 1H, J = 2 Hz); IR (neat, cm^{-1}) ν 2950, 1750, 1465, 1154.

2.3. Instruments

LC–MS/MS analysis was performed with a Micromass Quattro triple quadrupole mass spectrometer equipped with an electrospray ion source (Micromass UK Ltd., Altrincham, Cheshire, UK) and 1100 Modular HPLC System (Agilent Technologies, formerly Hewlett Packard, Winnersh, Berks, UK). Data acquisition was performed with Masslynx software (Micromass). Peak integration and calibration were carried out using Masslynx software (Micromass). Tecan RSP 5032 automatic liquid handler was purchased from Tecan Trading AG, Switzerland.

2.4. Preparation of calibration and quality control samples

To prepare calibration and quality control (QC) samples in urine, the appropriate volumes of stock and working solutions and acetonitrile (the total of acetonitrile added was 200 μl per sample) were pipetted into 100 μl of blank human urine using the liquid handler to yield varying urine concentrations of MSA and a fixed I.S. concentration (I.S. concentration approximately 100 μg/ml). Urine calibration standards were prepared to give a range of concentrations of approximately 1, 3, 8, 14, 23, 34, 47, 62, 80 and 100 μg/ml. Similarly, urine quality control samples at varying concentrations of 1, 3, 49 and 100 μg/ml were prepared from different primary standards (i.e., separate weighing) to those used for the preparation of the calibration standards.

Each spiked urine sample was then evaporated at 50 °C for 10 min. The dried sample was reconstituted in 100 μl of acetonitrile followed by addition of 100 μl of DBU in acetonitrile (0.15%, v/v) and 100 μl of *N*-(4-methanesulfonyl-benzoyl)-imidazole (0.3%, w/v). The sample was capped and swirled gently before standing at room temperature for 30 min to derivatize. Sample was evaporated at 50 °C under a stream of nitrogen for 10 min. The residue was redissolved in 1 ml of 30% acetonitrile, aq. and 4 μl was injected into the LC–MS/MS system for analysis.

2.5. LC–MS/MS conditions

Samples were delivered into the ESI source using a Luna 5 μ C18 column, 150 mm × 2 mm (Phenomenex UK Ltd., Macclesfield, Cheshire, UK) maintained at 40 °C. The mobile phase consisted of 1.25 mM ammonium formate buffer, acetonitrile and glacial acetic acid (89/11/0.011, v/v/v), with total running time of 17.5 min and flow rate of 0.4 ml/min.

The electrospray ionization (ESI) source was operated in the negative ion mode. Multiple reaction monitoring (MRM) at unit resolution was employed to monitor the transitions of the deprotonated molecular ions of derivatized MSA at m/z 276.2 → 197.2 and derivatized I.S. at m/z 290.2 → 211.2. Optimized MS parameters were: nebulizer and drying gas flow 60 and 600 L/h, respectively;

dwell time 2.00 s; IonSpray voltage −3000 V; electrospray block temperature 150 °C; desolvation temperature 450 °C; cone potential −30 V for both derivatized MSA and I.S.; extractor and RF lens voltage −5 and −0.5 V, respectively; collision energy −25 eV for both derivatized MSA and I.S.

2.6. Method validation

Quantification was achieved using derivatized MSA to I.S. peak area ratios. The concentrations of MSA in the QC samples were calculated by reference to the appropriate calibration curve using the method of $1/x^2$ weighted least squares linear regression and then compared to the theoretical (nominal) concentration. Peak area ratios were plotted against MSA concentrations and standard curves were in the form of $y = bx + a$. To evaluate linearity, urine calibration curves with calibration standards at 10 different concentrations prepared in duplicate were assayed for each of three validation occasions (analytical batches).

Similarly, to asses the intra- and inter-batch precision and accuracy of the method, six replicates of urine QC samples at four concentration levels were prepared and analysed for each of the three validation batches. The accuracy was expressed by (mean observed concentration − spiked concentration)/(spiked concentration) × 100 referred to as percent relative error (%RE) and the precision by percent relative standard deviation (%RSD). Intra-batch precision (repeatability) was estimated by the pooled intra-batch standard deviation of measured concentration values from the calculated run means whereas inter-batch precision (or intermediate precision) was estimated by the analysis of variance (ANOVA) based on the one-factor random effects model [4].

Derivatization yield combined with sample procedure recovery of MSA and the I.S. at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both derivatized MSA and I.S. with those obtained from direct injection of the derivatives dissolved in the processed blank urine.

Matrix effect was evaluated for blank human urine samples from six different subjects. The analyte and I.S. derivatives were continuously infused through a T-piece into the ion source to provide a background signal while injecting the processed blank urine on to the HPLC system to investigate the ion suppression effects of the matrix at the retention time of analyte and I.S. derivatives. Furthermore, six processed blank urine aliquots were spiked with MSA (at the LLOQ level) and I.S., analysed after derivatization and the results compared to the analysis after derivatization of a pure spike of analyte and I.S. in water at the same concentration levels, to demonstrate that no suppression had occurred.

Dilution analysis was performed by analysing one set of six QC samples at 250 μg/ml (approximately five times the mid-concentration samples). This set undergone one in five dilution using blank human urine to bring it into the calibration range, prior to analysis.

The long/short-term stability of stock solutions of MSA and I.S. was determined on storage at 5 °C and room temperature (69 days and 6 h, respectively). MSA stability in human urine was investigated using the low (2.91 μg/ml) and high (100 μg/ml) QCs in triplicate exposed to different time and temperature conditions. The short-term stability was assessed after storage of the test samples at room temperature for 4 h. The freeze–thaw stability was determined after three freeze–thaw cycles (−80 to 20 °C) on consecutive days. The stability of the derivatized solutions was assessed by placing QC samples at room temperature in the instrument over a period of 17 h. The results were compared with those QC samples freshly prepared, and the concentration percentage deviation was calculated.

2.7. Urine samples collection

Reparixin was administered by intravenous continuous infusion over a 48 h period to 27 adult male healthy volunteers (three cohorts, nine subjects per cohort) participating to a phase 1 ascending dose study (loading doses of 3.1, 3.8 and 6.8 mg/kg/h for 30 min, maintenance doses of 1.0, 2.0 and 4.2 mg/kg/h for 47.5 h, respectively). Urine samples were collected at −6/0 (predose), 0/48 and 48/60 h and stored at −80 °C until analysis.

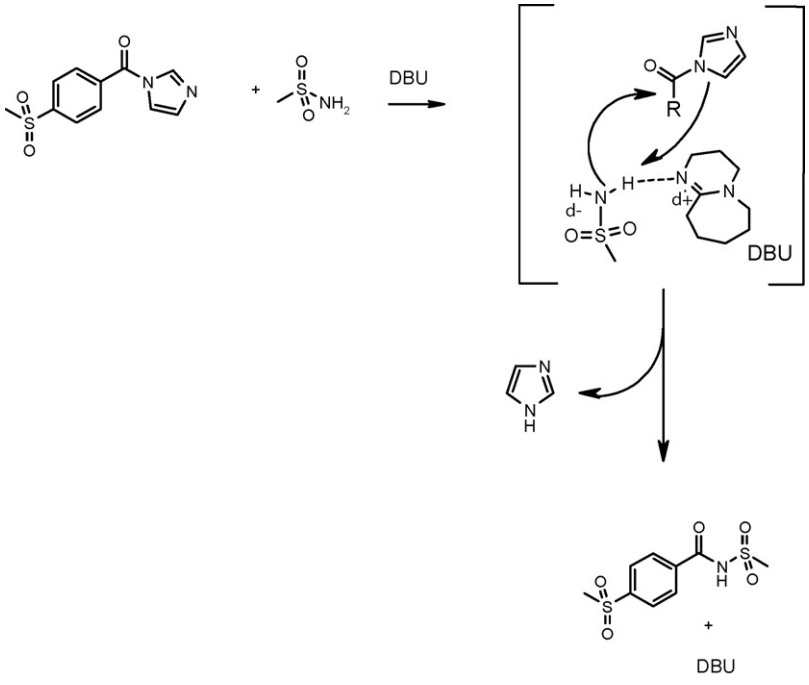

Fig. 2. Reaction scheme, derivatization of MSA by *N*-(4-methanesulfonyl-benzoyl)-imidazole.

3. Results and discussion

3.1. Derivatization and LC–MS procedures

The chemical structures of MSA and I.S. are shown in Fig. 1(B and C), respectively. As can be seen, ESA is the superior homologue of MSA and therefore a logical choice as an internal standard. Although an isotopic analog of MSA (for example, ^{13}C MSA) would be an ideal internal standard for this assay, it was not available at the time of validation.

As already discussed, due to the intrinsic physical chemical properties of MSA and ESA a direct separation and detection of these compounds from complex biological matrices, such as urine, is very difficult to obtain. With the purpose to obtain an acceptable chromatographic separation, a number of commercial derivatization reagents were unsuccessfully tried-out (see below), in the attempt to explore reactivity of the analyte and I.S.

Among the most common derivatization reagents suitable for compounds that contain active hydrogens, two different classes were tested: silylating and acylating reagents. Neither N,O-bis[trimethylsilyl]trifluoroacetamide (BSTFA), a silylating reagent, nor heptaflourobutyrylimidazole (HFBI), trifluoroacetylimidazole (TFAI), activated acylchlorides such as trichloroacethyl chloride or activated *N*-hydroxysuccinimides, all common acylating reagents, were able to properly react with either MSA and I.S. in the typical reaction conditions described in standard protocols, likely due to the marked chemical inertness of MSA and I.S. [5–9]. This is in agreement with literature data inasmuch as strong bases (NaH, NaOEt, etc.) are generally required to activate the sulfonamide group. Consistently, we have decided to set up a procedure for the MSA and I.S. derivatization in anhydrous conditions using DBU as a strong organic base [10,11]. We designed and synthesized a specific derivatizing reagent, *N*-(4-methanesulfonyl-benzoyl)-imidazole, and standardized the reaction conditions for the acylation of alkyl-sulfonamides. The reaction was carried out in anhydrous conditions, since the OH^- ions could compete with MSA and ESA that are weak nucleophiles. The proposed reaction scheme is depicted in Fig. 2.

The chemical structures of the MSA and I.S. derivatives are shown in Fig. 1E and F, respectively. As both derivatives have an acidic hydrogen (–NH–), negative ionization mode was chosen. Fig. 3A depicts a typical chromatogram of a patient's urine sample (MSA = 74.3 μg/ml), Fig. 3B depicts the chromatogram of drug-free urine sample and Fig. 3C depicts the chromatogram of an LLOQ spike in urine (1.00 μg/ml). The lack of a peak corresponding to MSA is noted. A clear chromatographic separation of the MSA derivative from the I.S. derivative can be seen from a comparison of Fig. 3A.

The run time was at least 18 min and the length of the chromatographic run depends on issues related to matrix effects. However, in our case it was not possible to significantly shorten the run time than was established, because unacceptable matrix effect problems, depending on the individual source of urine, were experimented.

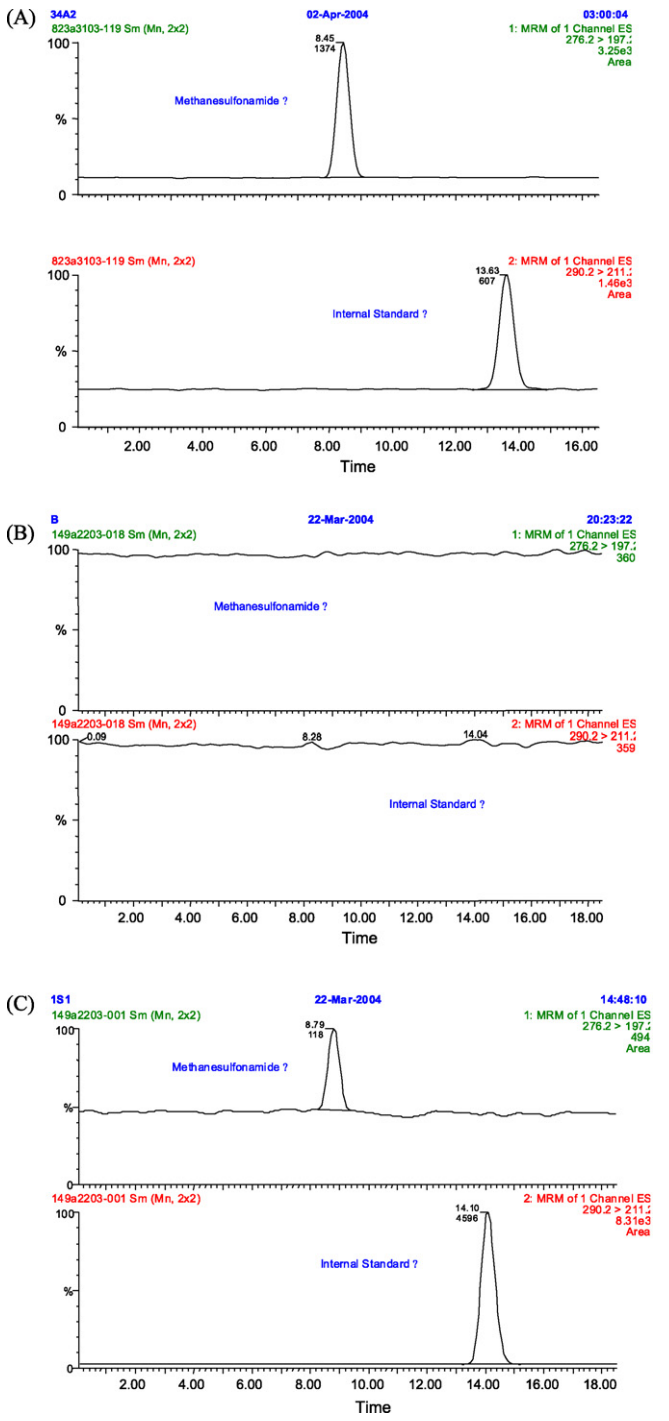

Fig. 3. (A–C): (A) LC–MS/MS chromatogram of patient's urine (74.3 μg/ml), (B) LC–MS/MS chromatogram of blank urine, (C) LC–MS/MS chromatogram of an LLOQ spike in urine.

3.2. Method validation

3.2.1. Matrix effects

There was no significant ion suppression/enhancement observed in the chromatograms of six processed blank human urine samples from six different subjects injected onto a background signal of either derivatized MSA or internal standard, at their retention times. Typical retention times for derivatized MSA and I.S. were about 8.5 and 13.6 min, respectively. Fig. 4 shows a typical chromatogram of a processed blank urine sample from a donor injected onto a background signal of both MSA and I.S. derivatives.

There was no significant difference in the peak area ratios of six processed blank urine samples spiked with MSA and I.S. derivatives (%RSD 6.7) compared to a pure spike of MSA and I.S. derivatives (%RSD 14.6) at the lower limit of quantification (1.00 μg/ml, LLOQ). The obtained accuracy was 123%.

3.2.2. Linearity of calibration curves and lower limit of quantification

Three consecutive calibration analyses were performed on different days with freshly aliquoted standards and the back calculated

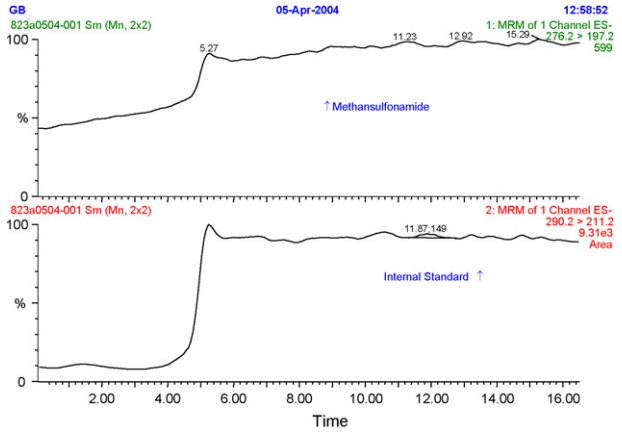

Fig. 4. Matrix effect chromatograms.

concentrations for each level evaluated (Table 1). The %RSD at each level varied from 2.1 to 7.2 and the %RE varied from −7.9 to 10.2 (acceptance limits were ≤15%, except at the LLOQ where a ≤20% limit was imposed, for both the %RSD and the %RE). The %RSD of the three slopes was 16.1 and the lowest coefficient of correlation (r) among the three calibration curves was 0.9954 (mean = 0.9970), being the acceptance limit >0.98.

Thus, the calibration curves did not exhibit any non-linearity within the chosen range. Since the back calculated results showed good day-to-day accuracy and precision and the visual inspection of the plotted duplicate calibration curves confirmed that the calibration curves were linear over the concentration range 1.0–100.0 μg/ml for the analyte, it was concluded that the MSA standard curve produced by this method could be used to reliably determine urine concentrations in a consistent fashion.

The LLOQ was defined as the lowest concentration on the calibration curve for which an acceptable accuracy of ±20% and a precision below 20% were obtained. The present LC–MS/MS method offered an LLOQ of 1.00 μg/ml in 100 μl urine sample.

The system suitability was verified before each validation occasion by calculating the precision (as defined by the %RSD) of the derivatized analyte to internal standard peak areas ratio, of three replicate injections of a suitable standard. All three system suitability tests complied with the acceptance specifications (%RSD ≤ 3%).

3.2.3. Precision and accuracy

Method accuracy, intra- and inter-batch precision was established and the results are summarized in Table 2. As can be seen, the assay was both accurate and precise between batches and within individual batches for each level. The greatest mean inter-batch accuracy (%RE) for MSA was +11.3% for the LLOQ (1.00 μg/ml, acceptance limit ≤20%). All non-LLOQ QC levels had inter-day percent deviations less than or equal to 5.5% (acceptance limit ≤15%). The method repeatability and intermediate precision (%RSD) for all QCs was less than or equal to 5.5% and 10.1%, respectively (acceptance limits were ≤15%, except at the LLOQ where a ≤20% limit was imposed, for both the within- and between-run precision). When the accuracy and precision of measuring MSA in diluted samples were assessed as described above, the mean %RE value for the 1:5 diluted samples was 8% (acceptance limits ±15%) and the %RSD was 6% (acceptance limit ≤15%), suggesting that should a sample have a concentration exceeding the upper limit of the calibration curve, the remaining urine sample could be diluted up to 5-fold and reanalyzed to fit within the established parameters.

3.2.4. Recovery and stability

The relative recoveries of MSA, determined at three concentrations (2.91, 48.84, 100.0 μg/ml), were 82 ± 5%, 100 ± 4% and 93 ± 12% (n = 3), respectively. The relative recovery of the I.S. was 62 ± 15% (n = 8).

The stability of MSA and I.S. stock solutions stored at 5 °C (±3 °C) over a 69-day-period was demonstrated with relative stabilities calculated at 92% and 95%, respectively (acceptance limits ± 10%); seemingly, the stability of MSA and I.S. stock solutions maintained at room temperature over a 6 h period was demonstrated with relative stabilities calculated at 103% and 100%, respectively (acceptance limits ±5%).

The stability of MSA in the urine matrix at two different QC levels (2.91 and 100.00 μg/ml) was also investigated as described above. Any deterioration of MSA during freeze–thaw cycles or extended time on the counter-top was monitored. Three freeze–thaw cycles (where the samples were completely thawed and either re-frozen or prepared for analysis) and one 4 h stability check at ambient temperature were performed. The stability of MSA in human urine taken through three freeze–thaw cycles was demonstrated, with recoveries of 107% at 2.91 μg/ml and 109% at 100.00 μg/ml (acceptance limits ±10%). The stability of MSA in human urine maintained at room temperature for 4 h prior to analysis was demonstrated, with relative stabilities of 108% at 2.91 μg/ml and 107% at 100.00 μg/ml (acceptance limits ± 10%). These results sug-

Table 1
Mean inter-day back calculated standard and standard curve results.

	Mean inter-day back calculated standard results										Standard curve results		
	Standard concentration (μg/ml)										Slope	Y-intercept	r
	1.00	3.20	7.61	14.31	22.76	33.81	47.47	62.43	79.99	100.14			
Validation Batch													
I	0.98	3.53	7.82	15.76	23.33	32.74	46.77	60.76	74.10	96.37	0.033163	0.0025934	0.9985
	0.97	3.22	8.01	15.02	22.25	34.30	47.77	60.93	76.39	94.25			
II	0.96	3.66	*	16.35	24.47	34.41	44.85	56.76	78.50	87.47	0.032705	0.013784	0.9954
	0.98	*	*	16.38	23.96	35.74	46.28	58.00	74.39	89.13			
III	0.86	3.36	8.04	15.75	23.55	34.27	46.41	61.42	75.02	94.30	0.024532	0.0046036	0.9972
	1.08	3.33	8.29	15.34	22.99	33.83	45.00	58.22	76.09	91.66			
Mean	0.97	3.42	8.04	15.77	23.43	34.22	46.18	59.35	75.75	92.20	0.030133	0.006994	0.9970
SD	0.07	0.17	0.19	0.54	0.77	0.97	1.10	1.93	1.62	3.41	0.0049	0.0060	0.0016
%RSD	7.2	5.1	2.4	3.4	3.3	2.8	2.4	3.3	2.1	3.7	16.1	NA	0.2
%RE	−2.8	6.9	5.7	10.2	2.9	1.2	−2.7	−4.9	−5.3	−7.9			
n	6	5	4	6	6	6	6	6	6	6	3	3	3

Note: Calibration curves were weighted 1/concentration^2. NA, not applicable.
* Calibration standard removed due to residual being outside set limits.

Table 2
Inter- and intra-assay accuracy and precision of MSA in urine.

Theoretical concentration of MSA (μg/ml)	Validation batch I	Validation batch II	Validation batch III	Intra-batch precision (%RSD)	Inter-batch precision (%RSD)	Average accuracy (%RE)
1.01	(LLOQ concentration level)			5.5	7.0	11.3
Mean	1.12	1.18	1.08			
SD	0.038	0.081	0.033			
%RSD	3.4%	6.9%	3.0%			
%RE	10.9%	16.8%	6.9%			
n	6	6	6			
2.91	(Low concentration level)			4.2	10.1	5.5
Mean	3.21	2.76	3.25			
SD	0.098	0.117	0.144			
%RSD	3.1%	4.2%	4.4%			
%RE	10.3%	−5.2%	11.7%			
n	6	6	6			
48.84	(Middle concentration level)			3.1	3.2	−0.4
Mean	49.58	48.09	48.32			
SD	0.905	2.283	0.840			
%RSD	1.8%	4.8%	1.7%			
%RE.	1.5%	−1.5%	−1.1%			
n	6	6	6			
100.00	(High concentration level)			3.8	6.9	−4.0
Mean	102.60	91.03	94.51			
SD	2.847	3.155	4.947			
%RSD	2.8%	3.5%	5.2%			
%RE	2.6%	−9.0%	−5.5%			
n	6	6	6			

gest that drug concentrations can be confidently determined in samples that had been previously thawed up to three times prior to the analysis or that have been thawed and kept at ambient temperature for up to 4 h. The 4 h stability test at ambient temperature was performed since the urine sample could conceivably stand on the bench for up to 4 h after thawing or before freezing.

The stability of derivatized MSA in the autosampler vials at three QC levels (2.91, 48.84 and 100.00 μg/ml) was also tested after 17 h at ambient temperature to allow for sample re-injection should a chromatographic or instrument malfunction occur during the initial analysis. The percent difference between the two sets of results was 4% or less, suggesting that there was no significant decline in the response for derivatized MSA during the 17 h storage in the instrument at ambient temperature.

3.3. Clinical application

The method was used to quantify MSA in human urine samples obtained from 27 healthy volunteers (nine subjects per group) receiving three different doses of reparixin for 48 h by continuous infusion (49.1, 96.9 and 202.9 mg/kg/48 h, respectively).

Mean MSA urinary excretion from 0 to 48 h was 66.2 ± 25.5 mg, 157.7 ± 68.3 mg and 312.0 ± 86.9 mg and from 48 to 60 h was 21.4 ± 9.7 mg, 35.5 ± 14.5 mg and 56.6 ± 56.7 mg, respectively. Cumulative urinary excretion from 0 to 60 h was 86.2 ± 30.9 mg, 191.0 ± 72.4 mg and 361.5 ± 123.0 mg, respectively, indicating that MSA urinary excretion increased linearly with the dose increase. Most of the metabolite was excreted within the first 48 h period (8–10%). The mean urinary excretion of MSA (0–60 h) accounted for 10.8 ± 3.5%, 12.3 ± 4.4% and 11.2 ± 4.2% of the reparixin administered doses, respectively.

4. Conclusions

In this article, we first developed a LC–MS/MS method to determine the concentration of MSA in human urine. This method combines the derivatization of MSA and I.S. by a novel reagent with the mass resolution and sensitivity of mass spectrometry. The method is rapid, specific and sensitive, which permits laboratory scientists with access to the appropriate instrumentation to perform MSA determination during pharmacokinetic or metabolism studies. The method was then successfully applied to the evaluation of urinary excretion of MSA in 27 healthy volunteers receiving different doses of reparixin by continuous intravenous infusion.

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