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A rapid LC–MS method for determination of plasma anion profiles of acidotic patients

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Abstract

In metabolic acidosis, the concentrations of anions associated with intermediary metabolism are increased and can make a significant contribution to the observed acidosis. Here we describe a method for the rapid determination of the plasma ultrafiltrate profile of these anions using liquid chromatography coupled to electrospray ionisation mass spectrometry (LC/ESI-MS). The ultrafiltrate from patients with acidosis resulting from various causes were examined and the results compared to control values. Using the LC/ESI-MS method described, a unique plasma ultrafiltrate anion profile was obtained for each of the groups studied that provides rapid diagnosis of the type of underlying acidosis.
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Keywords: Acidosis; Patients; Plasma; Ultrafiltrate; Krebs cycle; Anion gap; Intermediatry metabolism; Liquid chromatography; Mass spectrometry

1. Introduction

We have previously shown that the levels of anions normally associated with intermediary metabolism are altered in certain acidotic conditions and can contribute significantly to the development of the observed anion gap in these patients [1]. The absolute concentrations of these anions can be measured in a quantitative manner using enzyme assay. However, this method is slow and it can take several hours to determine the concentration of each individual anion. Gas chromatography or capillary electrophoresis coupled to a mass spectrometer have previously been used to examine anions in biological matrices but gas chromatography requires complex and time consuming chemical derivatisation of the anion(s) [2]; furthermore, by-products from this derivatisation have been reported to interfere with analysis. Other groups have reported the use of capillary electrophoresis to determine the anions present biological matrices, but this requires methodologically and technically demanding techniques in addition to time consuming extractions and chemical derivatisation [3]. Other authors have reported that if organic acids are to be measured in the serum of critically ill patients the measurement must be performed quickly due to the rapid metabolism of the anions [4].

With the increasing availability of robust liquid chromatographic separation coupled to on-line electrospray ionisation mass spectrometry (LC/ESI-MS) in hospital diagnostic laboratories we have developed a technique to allow rapid and routine determination of the anion profile in the plasma ultrafiltrate of patients. The determination of organic acids by HPLC has the advantage of being rapid, methodologically easier and more economical than many other techniques [5].

Although liquid chromatography of anions from biological matrices has been reported previously [5,6] these techniques have relied upon single wavelength UV detection coupled with the retention time to determine perturbations in the anion profile from a complex chromatogram. On-line LC/ESI-MS analysis, that would allow unequivocal identification of anions present in plasma has previously been regarded as impractical as a consequence of the presence of sulphuric acid in the mobile phase of ion exchange methods. We have developed a method utilising LC/ESI-MS that allows the unequivocal and rapid determination of the profile of relatively low molecular weight anions normally

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associated with intermediate metabolism from plasma ultrafiltrate and applied the findings to develop a method that allows the type of the underlying acidosis to be rapidly determined.

2. Experimental

This study was approved by the ethics committee of Guy's and St. Thomas' NHS Trust (Ref Number EC03/104). Prior to the sample being obtained, informed consent was obtained from the subject or where this was not possible, their next of kin.

2.1. Materials

All chemicals and solvents were of analytical or HPLC grade and were used without further purification. Unless stated otherwise, all the chemicals and solvents were purchased from Sigma Chemicals Ltd. (Poole, Dorset, UK). Enzymatic determination of the concentration of citrate, succinate, malate, D- and L-lactate acids levels in plasma ultrafiltrate were estimated using commercially available kits (Roche, Glasgow, UK). The levels of isocitrate and α-ketoglutarate were measured using our own enzyme assay utilising isocitrate dehydrogenase and α-ketoglutarate dehydrogenase respectively and their associated co-factors (Sigma Chemicals Ltd., Poole, UK). These assays are described elsewhere [1].

2.2. Instrumentation and chromatograpic conditions

We used an Agilent HPLC system (Agilent 1100) which consisted of a quaternary pump and on-line degasser coupled to a Series 1100 Mass-Spectrometer fitted with electrospray ionisation and operating in 'negative ion' mode (Agilent Technologies UK Ltd., Wokingham, Berkshire, UK). Purification of the anions in the sample was attained by use of an Aminex HPX-87H Ion Exclusion Column (300 mm × 7.8 mm, Bio-Rad, Hemel-Hempstead, Herts, UK). These columns were supplied from the manufacturers with the resin bathed in 0.008 M sulphuric acid. Since the presence of sulphuric acid in the ESI-MS suppresses analyte ionisation, it was removed prior to use by washing the column for approximately 150 h with mobile phase at a flow rate of 0.8 ml min^{-1}. Preliminary work had previously shown that the sulphuric acid could be replaced by HCl with no degradation of either column resolution or working life. Whilst in use the column was surrounded by a water jacket fed from a water bath maintained at 31 °C. Post-column, the eluent was split so that approximately 8% of the flow entered the electrospray nebuliser, the remainder being diverted to waste. This split was attained by use of a "T" piece and differing resistances to flow induced by the use of tubing of differing internal diameters (id) and length. The eluent was introduced to the "T" piece through PEEK tubing of 0.75 mm id. The arm that split the flow to waste consisted of approximately two metres of PEEK tubing of 0.25 mm id. The arm that carried the flow to the nebuliser consisted of a piece of PEEK tubing 14 cm long of 0.75 mm id.

Prior to entering the MS source the HCl in the stream was partially neutralised by the addition of 10 mM ammonium acetate in a 50:50 (v/v) methanol/water mixture at a flow rate of 0.09 ml h^{-1} through a second "T" piece. The eluent was then introduced to the ESI source of the MS via 8 cm of PEEK tubing of 0.25 mm id (outlined in Fig. 1).

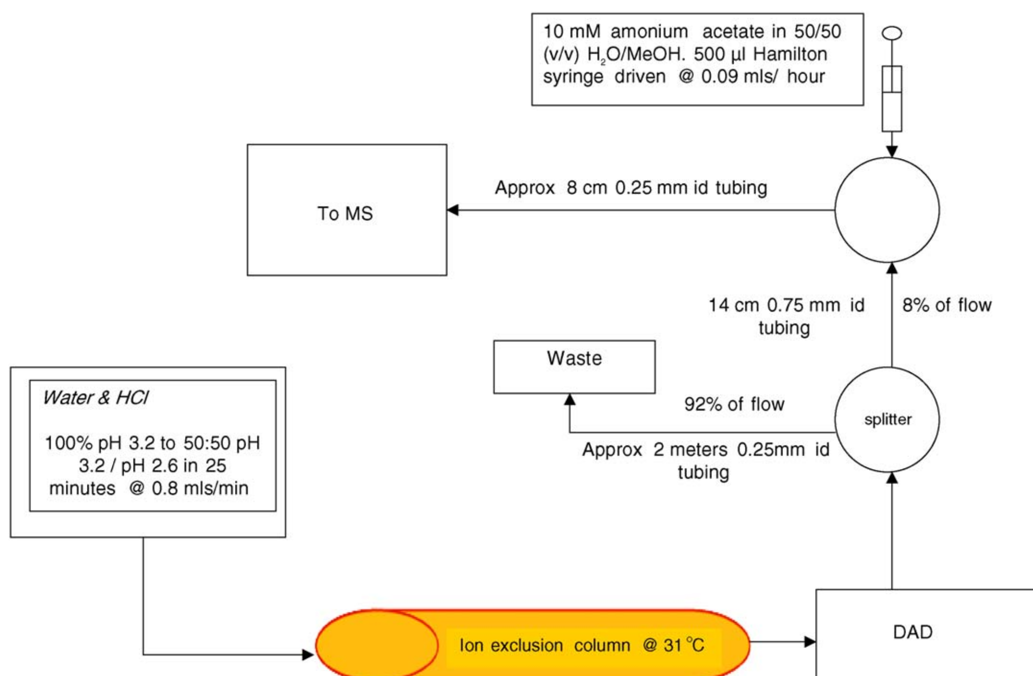

Fig. 1. Schematic layout for the on-line measurement of anions usually associated with intermediate metabolism in human plasma ultrafiltrate using liquid chromatography coupled with negative ion electrospray mass spectroscopy.

2.3. Sample preparation—enzyme assay

Patient studies were undertaken on 15 ml of blood taken from arterial cannulae in patients with metabolic acidosis whose standard base deficit was 8 mmol l^{-1} or greater. Control samples were obtained from venous blood of laboratory workers. Due to its associated risk, arterial puncture was deemed inappropriate in the control group. The blood was drawn into a non-heparinised syringe before being rapidly transferred into SST II (KODAK) Vaccutainers (BD Vaccutainer Systems Ltd., Plymouth, UK).

Once obtained, the sample was chilled and rapidly transported to the laboratory. Plasma was isolated by centrifugation of the Vaccutainers (1500 × *g*) at 4 °C for 10 min. The plasma was transferred to an Amicon 30,000 Da cut-off filter (Millipore, Herts, UK) where centrifugation at 1560 × *g* for 15 min produced ultrafiltrate. The ultrafiltrate was either immediately analysed or stored at −20 °C for analysis within 24 h. Previous work had highlighted the need for rapid assay of the samples due to an observed rapid decrease in concentrations of the measured anions in frozen plasma, and an appreciable though slower decline in frozen plasma ultrafiltrate.

Oxaloacetate could not be measured by either enzymatic techniques or LC–MS as a result of its short half-life (approximately 69 s) in aqueous systems [7].

2.4. Sample preparation—LC–ESI/MS

Mobile phase was prepared by adding 270 μl of concentrated (10.56 M) HCl to 1000 ml of water. Twenty microliters of the plasma ultrafiltrate was withdrawn and diluted 1:9 (v/v) with mobile phase before being passed through a 0.22-μm filter to remove any precipitate. LC/ESI-MS analysis was performed on 200 μl of this diluted, filtered material.

2.5. LC–ESI/MS sample fractionation

Two hundred microliters of the diluted plasma ultrafiltrate was separated on the LC system using a mobile phase that ramped linearly from water/HCl at pH 3.2 to water/HCl at pH 2.6 over 25 min. The conditions in the mass spectrometer were as follows: Nebuliser—nitrogen gas at a temperature of 300 °C was introduced at a rate of 5 l min^{-1} to give a nebuliser pressure of 25 psig. MSD—the fragmentor potential was set to 70 V and the ion energy was 5 V. The HED was 10,000 V.

2.6. Determination of retention time and assigning peak identity

Retention times of the various acids were determined in water and also in human plasma ultrafiltrate by spiking with individual standards of citric, fumaric, pyruvic, succinic, acetoacetic, lactic, 3-OH-butyric, α-ketoglutaric and malic acid to a final concentration of 0.45 mM. Once the individual retention times were established, the acids were chromatographed as a mixture. Standards were prepared freshly before use. When viewed as extracted ion currents, this allowed unequivocal identification of the peaks resulting from each anion.

When viewing the total chromatogram obtained from patient samples it was difficult to resolve that portion of the peak resulting from succinate and that portion which was due to lactate. By use of the extracted ion current we were able to overcome this problem and describe the anionic profiles in the various patients groups. Further difficulties can be encountered when trying to assign peaks resulting from the presence of citrate and isocitrate since these anions are isomers and share the same mass and very similar retention times. This problem can be overcome by exposing the sample to isocitrate dehydrogenase and its associated co-factors prior to fractionation. This eliminates the component of the peak caused by isocitrate and its absence thus allows unequivocal identification. However, using this enzyme degradation technique increases the time taken to assay a sample.

3. Results

All data are presented as mean ± standard deviation. Unless stated otherwise, the data are normally distributed and statistical analysis was undertaken with an unpaired *t*-test. Where the data were not normally distributed a Mann–Whitney non-parametric test was applied. In both cases, significance was deemed to have been attained if *p* was less than, or equal to 0.05.

For the enzyme assay, samples were obtained from 13 patients with diabetic keto-acidosis and five patients with lactic acidosis. Five patients had a metabolic acidosis that could not be ascribed to either lactic acidosis, keto-acidosis or an exogenous agent. Five patients had an acidosis as a result of gastrointestinal or renal anion loss. Twelve volunteers donated blood for the control samples. Due to the constraints of obtaining samples from critically ill patients in acute clinical medicine, not all samples were adequate for both enzymatic and mass spectrometric assays. For on-line LC/ESI-MS, samples were analysed from nine patients with diabetic keto-acidosis, five patients with lactic acidosis, five patients had an acidosis that could not be ascribed to either lactic acidosis, keto-acidosis or an exogenous agent. Seven patients had an acidosis as a result of gastrointestinal or renal ion loss. Seven volunteers donated blood for the control samples.

Using the LC–ESI/MS method described, α-ketoglutarate had a retention time of 6.77 ± 0.16 min, citrate had a retention time of 7.19 ± 0.07 min, malate 8.26 ± 0.59 min, succinate 10.39 ± 0.13 min and lactate a retention time of 10.63 ± 0.12 min.

Typical extracted ion currents obtained from the various study groups are shown.

Fig. 2 shows a typical extracted ion current from a control individual.

Fig. 3 shows a typical extracted current from a patient with a diabetic ketoacidosis.

Fig. 4 shows a typical extracted ion current for a patient with a lactic acidosis.

Fig. 5 shows a typical extracted ion current for a patient with an acidosis of unknown origin.

Fig. 6 shows a typical extracted ion current for a patient with an acidosis with a normal anion gap.

The mean concentrations of the anions measured enzymatically in each of the above groups are shown in Table 1. The

Table 1
Concentrations of anions in plasma ultrafiltrate measured enzymatically (μEq/l)

Anion	Patient group													
	Diabetic ketoacidosis			Lactic acidosis			Unknown origin			Normal anion gap			Control	
	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.
Citrate	454.02	194.11	ns	1453.13	513.95	<0.01	335.63	69.82	ns[a]	239.1	105.1	ns	448.55	119.80
Isocitrate	421.93	352.56	0.02	704.6	347.6	<0.01	949.16	883.22	<0.01[a]	84.48	69.6	ns[a]	60.97	31.31
α-Keto	413.41	158.48	<0.01[a]	547.72	344.98	<0.01[a]	651.51	203.06	<0.01[a]	72.97	67.34	ns[a]	79.17	106.74
Succinate	181.1	173.24	ns	358.27	112.49	<0.01[a]	340.04	128.74	0.02[a]	125.89	73.57	ns[a]	90.29	49.97
Malate	229.81	181.87	<0.01[a]	593.65	265.88	<0.01	485.16	189.67	<0.01[a]	95.07	117.59	ns	59.82	32.94
D-Lactate	157.34	67.85	<0.01	397.69	511.15	<0.01[a]	176.49	135.21	<0.01	69.27	55.39	<0.01	35.63	18.42

For each group, statistical analysis is presented relative to control value.
[a] Mann–Whitney non-parametric test. ns, not significant; S.D., standard deviation; α-keto = α-ketoglutarate.

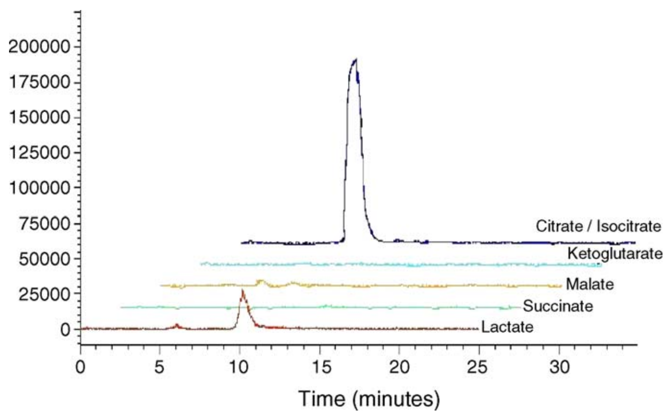

Fig. 2. Control.

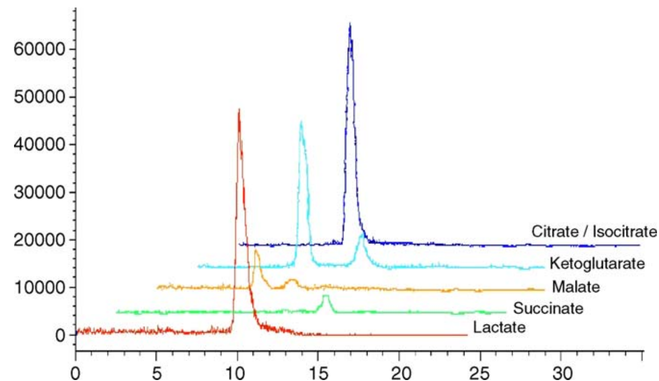

Fig. 3. Diabetic ketoacidosis.

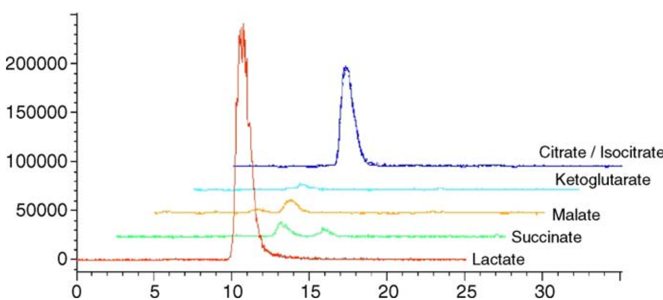

Fig. 4. Lactic acidosis.

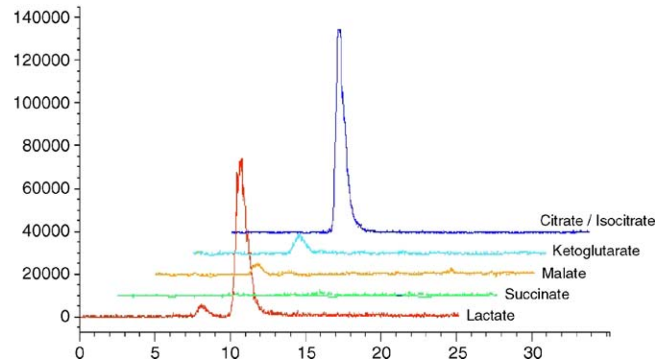

Fig. 5. Acidosis of unknown origin.

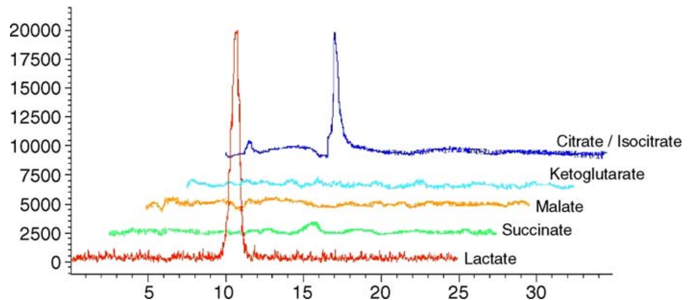

Fig. 6. Normal anion gap acidosis.

area under the curve (AuC) obtained from peaks of each of the extracted ion current of the LC–ESI/MS for the anions in each of the above groups are shown in Table 2.

4. Discussion

Acid/base homeostasis is crucial in health and in critical illness a disturbance in this balance is often a marker of poor prognosis. Despite this, in clinical settings only lactate is routinely measured. We have previously shown that, in certain acidotic states, the level of circulating anions normally associated with intermediary metabolism are elevated and make a significant contribution towards the anion load that has previously been overlooked [1]. Although enzymatic methods exist to measure the absolute quantities of anions in the plasma, these methods are slow, cumbersome and provide a degree of accuracy that

Table 2
Area under curve for extracted ion currents of anions in plasma ultrafiltrate

Acid	Patient group													
	Diabetic ketoacidosis			Lactic acidosis			Unknown origin			Normal anion gap			Controls	
	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.	*p*	Mean	S.D.
Lactate	1754508	699743	<0.01	6511084	5001468	<0.02	1567258	976921	<0.02*	823421	491095	ns	594126	382388
Succinate	243939	170204	<0.01*	358556	373346	ns[a]	88516	32991	ns*	38628	22999	ns[a]	69936	65577
Malic	80369	60526	ns*	414671	241390	0.04[a]	207324	101513	0.02	96926	147412	ns*	80369	60526
Ketoglutaric	843542	391793	<0.01*	135119	76328	0.03	155241	127644	ns[a]	53516	51467	ns*	42919	34005
Citrate/isocitrare	1794828	1327456	ns	3721674	2314098	ns	1572625	1374111	ns	554106	540187	0.01*	2483098	2460457

For each group, statistical analysis presented relative to control value 'ns' denotes not significant.
* Mann–Whitney non-parametric test.
[a] Unpaired *t*-test, Welch corrected.

is very often unnecessary in clinical settings where a measurement of the absolute amount of an anion is often of secondary importance to its relative amounts and/or presence. In this paper we have described a method utilising ion exchange liquid chromatography coupled to mass spectrometry that allows a rapid assessment of the anion profile in acidotic patients.

Using the LC/ESI-MS method described, a unique anion profile was obtained for each of the groups studied. Patients with diabetic keto-acidosis, in addition to the anticipated gross elevations of hydroxybutyrate and acetoacetate, generally exhibited significant elevations in their levels of lactate, succinate and α-ketoglutarate compared to control values. Patients with lactic acidosis generally exhibited significantly increased plasma ultrafiltrate concentrations of malate and α-ketoglutarate relative to controls as well as markedly elevated concentrations of (L-) lactate. Patients with acidosis of unknown origin generally showed increased levels of malate and more minor increases in lactate relative to controls whilst patients with normal anion gap acidosis showed no significant increase in either lactate, succinate, malate or α-ketoglutarate. Concentrations of citrate did not generally differ from that of control values except in patients with normal anion gap acidosis where a significant decrease in citrate was observed.

Examining the results from the enzyme assay shows that patients with keto-acidosis when compared to controls demonstrated significantly elevated levels of isocitrate, α-ketoglutarate and malate compared with controls. Patients with lactic acidosis showed elevated levels of citrate, isocitrate, α-ketoglutarate, succinate and malate compared with controls. Patients with acidosis of unknown origin showed significant elevations in the levels of isocitrate, α-ketoglutarate, succinate and malate compared to controls, whilst patients with normal anion gap acidosis showed no significant changes in the levels of any of these anions compared to control levels.

These findings can legitimately be criticised for the small number of patients studied in each group bearing in mind the frequency with which patients with metabolic acidosis are seen in a large acute hospital. There are numerous problems in obtaining experimental samples from acutely ill patients, the main difficulty being that the effort of the attending physicians was, quite correctly, directed towards the resuscitation of the critically ill patient, and not to obtain samples for an experimental study. Sample acquisition problems were compounded by the requirement of ethical consent for each of the samples taken and very often the attending physician thought it inappropriate to seek consent from a distressed relative of the critically ill patient. We expended considerable effort in acquiring samples and the results presented here represent the total of all the samples obtained with consent from acidotic patients in excess of a period of two years.

It must be pointed out that the concentrations of several of the anions measured in this study did not reach significance when compared with controls despite, on first glance, appearing to be significantly elevated. This was a probably a result of both the restricted size of the populations studied and of the large standard deviation of the data, resulting from the heterogeneity of the patients in the study and the differing levels of severity in the underlying cause of the acidosis. No matter how the anions are measured, the contribution that they make towards the total acidosis is greater than that which may be initially apparent. With the partial exceptions of citric and isocitric acid (which are 97% ionised at pH 7.0), the acids examined in this study are effectively fully ionised at the measured pH. Unlike lactic acid, not all are monobasic. Citric and isocitric acid are tribasic and contribute three protons, whilst α-ketoglutaric, malic and succinic acid are dibasic and contribute two protons to the solution on ionisation. The contribution to the anion gap made by these anions is thus not insignificant.

We have not attempted to produce absolute values for the anions when measured by LC–ESI/MS by comparison with a calibration curve as a result of differential ionisation. Even though the sample analysed was composed of plasma ultrafiltrate, it inevitably contained numerous other compounds some of which shared a similar retention time with the plasma acids. Since the samples for this study were obtained from different patients suffering from a variety of diseases, the composition of the plasma ultrafiltrate matrix was not consistent but showed heterogeneity between patients. When numerous compounds enter an electrospray ionisation source they compete for the available charge. Since the flow entering the ionisation source contained within its matrix compounds whose absolute amounts varied between patient samples, the absolute composition of the flow entering the nebuliser could not be determined with certainty. Being unsure of the suppression each of the anions

were experiencing in the different patient samples we prefer to refer to acid profiles rather than absolute amounts. UV spectrometry cannot overcome this difficulty as often the co-eluting substance also possesses a structure that absorbs in the area of the UV spectrum monitored. However, using LC–ESI/MS, complete resolution of the plasma acid profile is not required to obtain a satisfactory 'fingerprint' that is indicative of the underlying aetiology of the acidosis.

Given the differential ionisation it is perhaps unsurprising that the anion profiles differ between those obtained by MS and those obtained from enzyme assay. Nonetheless, it appears that the anion profile obtained by LC–ESI/MS for each type of acidosis is virtually unique to the underlying condition causing acidosis and may thus provide the clinician with an aid to rapid diagnosis of that condition.

We are unaware of any previous work examining in detail the role of anions usually associated with intermediate metabolism in various acidotic states. Haas et al. [6] and Lippe et al. [5] have both produced limited data on certain anions in plasma extracts using liquid chromatography coupled to on-line UV spectrometry. Haas et al. examined certain anions in a plasma extract of control individuals and in one patient suffering from lactic acidosis. Their results confirm the elution order of the anions from the Aminex column that we report here. A difference in the retention times is observed between the two studies, but the earlier work used two Aminex columns in series, whilst the results obtained in this paper were obtained with a single column. In addition to reporting the plasma profile of certain anions in human plasma, Haas et al. reported the levels of α-ketoglutatrate, citrate, lactate and 3-hydroxybutyrate in one patient with lactic acidosis. In this patient, α-ketoglutatrate increased by approximately five fold, citrate by two fold, lactate by 12 times and β-OH butyrate by three times relative to control values. In our study, the enzyme assay reported that the level of α-ketoglutaric acid increased significantly by seven times, whilst that of citrate increased three times relative to control values, although this increase in citrate did not attain significance. When examined by LC–ESI/MS the levels of α-ketoglutaric acid in our study increased significantly by a factor of three times and lactate significantly increased by eleven times. Although the level of citrate measured by LC–ESI/MS increased two fold it again did not attain significance. The results obtained from lactic acidotic patients in this study are therefore similar to the previously published limited data obtained from one patient.

Lippe et al. [5] used liquid chromatography coupled to a UV spectrophotometer to examine organic acids in the plasma of patients hospitalised for an assessment of the metabolic control of diabetes and compared these to normal controls. They reported that in the diabetic population the levels of citrate had doubled whilst the level of hydroxybutyrate had increased fourteen fold when compared to their control population. In our study we found that the level of hydroxybutyrate increased significantly by almost twenty times in those with diabetes, but that the level of citrate did not differ significantly when compared to control values. It is widely reported elsewhere that plasma citrate is generally not elevated in acidosis [8,9], and we confirmed this in a previous study [1] as well as the results reported above.

Other authors who have analytically profiled components of physiological fluids have relied upon gas chromatography after chemical extraction. These techniques have been criticised since solvent extraction has been reported to show poor reproducibly and efficacy [10], whilst formation of the suitable derivatives necessary for gas chromatography is not easy to perform and the by-products may interfere with the analysis [6]. Capillary electrophoresis coupled to mass spectrometry has been described by other groups but use of these instruments is methodologically and technically demanding. However, a relatively recent report has described successful fractionation of anionic intermediates in *Bacillus subtilis* using CE coupled to ESI/MS [11].

With the advent of readily available, robust and relatively cheap single quadrupole electrospray mass spectrometers in hospital diagnostic laboratories, sample analysis that once existed solely in the realm of specialist diagnostic centres is becoming commonplace and routine. The information obtained by application of the LC–ESI/MS technique described in this paper was, when rapidly returned to the clinicians, of immediate value in the management of patients with metabolic acidosis. Indeed, using this technique, we twice identified clinically unexpected diabetic keto-acidosis in patients that had arisen from a combination of diabetes and inadequate nutrition, resulting in a near normal blood glucose levels. Since the concentration of 3-OH-butyric acid is currently seldom determined it is questionable whether this would have come to light by any other generally used investigation. The method described is flexible and could easily be applied to monitor perturbations in the level of other compounds whose distribution is disturbed in plasma ultrafiltrate in other metabolic diseases.

5. Conclusion

By utilising the method described we have developed a technique that allows rapid determination of the anion profile in the plasma ultrafiltrate of acidotic individuals without the requirement of chemical derivatisation and using widely available and robust electrospray ionisation mass spectrometry. The data obtained suggests that each of the acidotic groups studied in this paper possessed its own unique anion profile which can be used to identify the underlying cause of the acidosis. Even in this limited series, the anion profile information obtained by LC–ESI/MS was, when rapidly returned to the clinicians, of real value in the management of the vast majority of the cases of simple lactic acidosis, "unexplained" acidosis, or on several occasions, an unrecognised diabetic-type keto-acidosis. The technique described is flexible and can easily be used to examine perturbations in the level of other low molecular weight compounds whose distribution is disturbed in plasma ultrafiltrate in other metabolic diseases.

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References

[1] L.G. Forni, W. McKinnon, G.A. Lord, D.F. Treacher, J.M. Peron, P.J. Hilton, Crit. Care 9 (5) (2005) R591.
[2] H.M. Liebich, A. Pickert, B. Tetschner, J. Chromatogr. 289 (1984) 259.
[3] P. Daish, J.V. Leonard, Clin. Chem. Acta 146 (1985) 87.
[4] H.-J. Guth, M. Zschiesche, E. Panzig, P.E. Rudolph, B. Jager, G. Kraatz, Int. J. Artif. Organs 22 (1999) 805.
[5] G. Lippe, R. Trevisan, R. Nosadini, R. Fabris, R. Deana, Clin. Biochem. 20 (1987) 275.
[6] R.H. Haas, J. Breuer, M. Hammen, J. Chromatogr. 425 (1988) 47.
[7] C.S. Tsai, Can. J. Chem. 45 (1967) 873.
[8] E.C. Rackow, C. Mercher, M.E. Astiz, C. Goldstein, D. McKee, H. Weil, Circ. Shock 30 (1990) 107.
[9] C. Mecher, E.C. Racow, M.E. Astiz, M.H. Weil, Crit. Care Med. 19 (1991) 707.
[10] J.A. Thompson, S.P. Markey, Anal. Chem. 47 (1975) 1313.
[11] T. Soga, Y. Ueno, H. Naraoka, Y. Ohashi, M. Tomita, T. Nishioka, Anal. Chem. 74 (2002) 2233.