Near infrared reflectance spectroscopy preliminary study on the N-oxidation of sparteine in man

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Introduction

Sparteine, an antiarrhytmic drug, is metabolised by N-oxidation. The sparteine-N_1-oxide rearranges with loss of water to 2- and 5-dehydrosparteine (Figure 1). About 5% of the population is unable to metabolise the drug.[1] These persons, who were designated nonmetabolizers, excreted almost 100% of the administrated dose in urine as unchanged drug (Figure 2). The ratio of sparteine/metabolites reflects the capacity of the individual to metabolise the drug. The defective metabolism of sparteine was found to have a genetic basis.

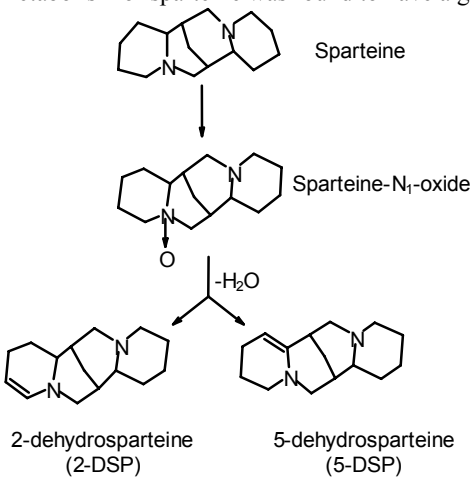

Figure 1. Metabolic pathway of sparteine in man.

Liquid products can be analysed directly by using cuvettes, fibre optics probes etc. Numerous successful studies have been reported, using Dry Extract Spectroscopy by Infrared Reflection (DESIR) in medical applications. Diagnostic prediction of DESIR was considered as a potential method for detecting breast cancer.[2] The urea concentration in dried human serum was determined by DESIR and MLR.[3]

The aim of the present study was to elaborate the technique DESIR as a method to measure the amounts of sparteine and its metabolites in urine.

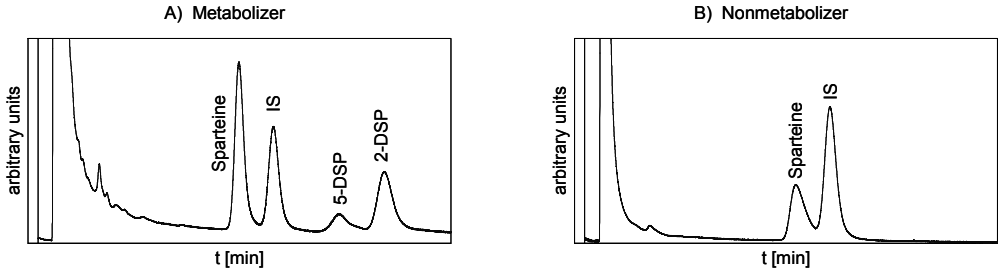

Figure 2. Gas chromatograms of urine extract.

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Materials and methods

160 patients with normal kidney and liver function, fasted overnight and then took sparteine sulphate (100 mg) in the morning after emptying the bladder. Urine was collected for up to 12 h. Urine samples were stored at –20°C and then analysed by gas chromatography for sparteine and its metabolites. Urine (2 mL) and the internal standard solution (0.05 mL) were pipetted into a tapered tube, and 0.2 mL NaOH solution and 0.5 mL dichloromethane were added. The sample was mixed on a rotary mixer. After centrifugation 0.001 mL of organic phase was injected onto the column.[4] All chemicals used were of analytical grade. An Elwro type 504M gas chromatograph (Elwro, Wroclaw, Poland) with FID detector was used. Quantitation (KSPD software ver.4.0, Metroster, Torun, Poland) was done by use of the peak area ratios of sparteine and its metabolites to the internal standard. A linear relationship was obtained for the range tested (0.05–200 mcg mL^{-1}). The precision of the chromatography method was good, with a coefficient of variation of 4%.

Glass fibre filters (GF/A, 47 mm, Whatman International Ltd., Maidstone, England) were dried at 60°C for 15 min. Aliquots of 1 mL urine samples were pipetted on the centre of the filters. The filters were dried at 60°C and stored for 2 h in a desiccator, before NIR measurements. The non-volatile organic analytes (sparteine and its metabolites) extracted from the urine samples remaining on glass fibre filters were measured by InfraAlyzer 500 spectrophotometer (Bran+Luebbe GmbH, Norderstedt, Germany) in diffuse reflectance mode. Each sample was applied on duplicate filters. Reflectance spectra were measured in triplicate on each filter in the wavelength range between 1100 and 2500 nm at 2 nm intervals. To obtain calibration equations, a multiple linear regression (MLR) was carried out between the spectral data and sparteine, 5- and 2-dehydrosparteine concentrations obtained using gas chromatography method. Data were processed by Sesame software ver.2.10 (Bran+Luebbe GmbH, Norderstedt, Germany). These calibrations were then applied to a separate set of 48 urine samples which, for validation purposes, were also analysed by gas chromatography method.

Results and discussion

The purpose of this study was to assess the feasibility of DESIR analysis of sparteine and its metabolites in man urine samples. After removal of few outlier points – that possibly resulted from errors in the gas chromatographic analysis – the calibration of sparteine, 5-dehydrosparteine and 2-dehydrosparteine concentrations in urine, using MLR method and the second derivative of log (1/*R*), was of high predictive value (R^2 = 0.97; 0.91; 0.94). Based on Table 1, it can be concluded that after validation of the calibration equations, good agreement was observed between the results of the chromatography method and those of the DESIR method for all analytes (the squares of correlation coefficients were 0.94, 0.89 and 0.91 for sparteine, 5-dehydrosparteine and 2-dehydrosparteine, respectively). Calibrations based on the first derivative of log (1/*R*) were of lesser predictive value.

Table 1. Calibration and validation statistics obtained for the sparteine and its metabolites extracted from the urine samples on the glass fibre filters.

Constituent (mg)	Calibration data (n = 160)					Validation data (n = 48)				
	Range	Mean	*SD*	*SEC*	*RSQ*	Range	Mean	*SD*	*SEP*	*RSQ*
Sparteine	2.02–49.50	18.55	10.68	0.24	0.97	2.54–42.69	18.18	10.44	0.27	0.94
5-DSP	0.00–13.24	2.55	1.92	0.19	0.91	0.00–12.57	2.49	1.85	0.26	0.89
2-DSP	0.00–50.40	11.62	8.34	0.17	0.94	0.00–33.26	10.76	6.66	0.20	0.91

The present study showed that the spectra of urine at the wavelength region of 2100–2300 nm was highly correlated with sparteine and its metabolites concentrations (Figure 3).[5] Based on the reference assignments of bands in NIR regions, that region represents N-H bond component.[6]

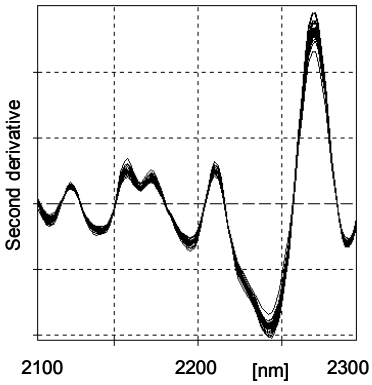

Figure 3. Second derivative treated spectra of glass fibre filters with analytes extracted from the urine samples (region 2100 – 2300 nm).

This model study indicated that DESIR has a potential as a cost-effective screening method. Compared with gas chromatography, DESIR is much faster, more economical and needs no chemicals. Compared to direct NIR measurement, DESIR is somewhat slower, but gives opportunities to measure compounds that are present in low concentration (sparteine and its metabolites in man urine samples). In the next stage of our investigations, prior to deposition onto the glass fibre filters a solid phase extraction (SPE) will be used to improve the results. In this way the analytes will be purified and concentrated within few minutes.

Acknowledgements

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References

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