Poster Session 2 – Analytical Chemistry

083

Simultaneous spectrophotometric determination of a ternary mixture, ambroxol HCl, theophylline and guaifenesin in capsules using chemometrics

A. M. Wahbi, O. Fahmy and M. Moneeb

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt. E-mail: Abdel_azizwahbi@yahoo.com

Ambroxol HCl (I) 30 mg, theophylline (II) 60 mg and guaifenesin (III) 100 mg are formulated together in the form of Trisolvin capsules for the treatment of respiratory disorders. In 0.1 M HCl, the three compounds possess maximum absorption at 245, 270 and 273 nm, respectively, with considerable overlapping. Multivariate spectrophotometric methods have been developed for the simultaneous determination of this mixture using: absorbance data, $A_{mi} = a_{Ii}\ C_I + a_{IIi}\ C_{II} + a_{IIIi}\ C_{III}$; absorbance ratio data, $A_{mi}/a_{IIIi} = (a_{Ii}/a_{IIIi})\ C_I + (a_{IIi}/a_{IIIi})\ C_{II} + 1x\ C_{III}$; and derivative (first- and second-order) data, $D_{mi} = \delta_{Ii}\ C_I + \delta_{IIi}\ C_{II} + \delta_{IIIi}\ C_{III}$, where m, i, a, and δ stand for mixture, ith wavelength, specific absorbance and specific derivative value, respectively. The absorbance of the standard and sample solutions were recorded over the wavelength range 230–300 nm at 2-nm intervals. The derivative data were generated from the absorbance data using a BASIC program. The absorbance ratio curves of I and II divided by III were found to possess maxima at 243 and 281 nm, respectively. PLS models were constructed using the training set together with the Chemometrics Toolbox 3.02 software with Matlab (Kramer 1998). In construction of the training set, the experimental design was adopted to maximize statistically the information content in the spectra. The samples in the training set were designed to cover the range of interest and appear to be well distributed within space (Kenneth et al 1998). The PLS models constructed using three factors succeeded to span nearly all the data leaving only negligible residuals. Validation sets were used to determine the three drugs in mixtures. The mean percentage recoveries were found to be within ±1% of the claimed concentrations. Trisolvin capsules have been analysed by the proposed functions (Table 1). The specificity of the studied PLS method has been tested. The results obtained for the analysis of laboratory prepared mixtures and Trisolvin capsules using the absorbance ratio data were found to be closely similar to those obtained using absorbance data. This confirms specificity of the proposed method for the three components present in this ternary mixture. The presence of any absorbing foreign substance in the test solution will lead to different results when using absorbance and absorbance ratio data. The simultaneous determination of I, II and III has also been carried out by PLS using first- and second-order derivative data. The results obtained were found to be closely similar to those obtained using absorbance data. This confirms that there was no interference in the sample solutions from capsule excipients. The PLS method using absorbance data is manifest in being fast and simple and does not require prerequisites for successful application. It is a one-step calculation to obtain all the unknown concentrations.

Table 1 Spectrophotometric determination of ambroxol HCl, theophylline and guaifenesin in Trisolvin* capsules using PLS

	Percentage found*			
	A	AR[1]	D[2]	D
Ambroxol	99.3 (0.34)	99.2 (0.31)	99.1 (0.36)	99.2 (0.33)
Theophylline	100.3 (0.21)	100.3 (0.26)	100.2 (0.29)	100.1 (0.27)
Guaifenesin	99.7 (0.32)	99.8 (0.33)	99.6 (0.27)	99.5 (0.30)

A = absorbance, AR = absorbance ratio, D = derivative.
*Mean percentage found of six different concentration ± RSD%.

Kenneth, R. B. et al (1998) *Chemometrics. A practical guide*. New York: John Wiley and Sons Inc.

Kramer, R. (1998) *Chemometric techniques for quantitative analysis*. New York: Marcel Dekker Inc.

084

Achieving high accuracy and precision in LC-MS/MS assays for pharmaceutical analysis

L. N. Alfazema[1,2], D. S. Richards[2], S. Gélébart[2], J. C. Mitchell[1] and M. J. Snowden[1]

[1]Medway Science, University of Greenwich, Medway Campus, Chatham, Kent ME4 4TB, UK and [2]Analytical R and D, Pfizer Global Research & Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK. E-Mail: Louisa.Alfazema@pfizer.com

The development of accurate and precise LC-MSMS assays in pharmaceutical industry requires an understanding of the origin and the extent of errors that will affect measurements. In LC-MSMS, errors can be derived from several sources, including integration, HPLC injection volume, variation in detector response and ion suppression. Improved chromatographic peak shape, use of internal standards, suitable linear range and co-elution of internal standards, respectively, can be used to address the above errors. Sample preparation steps can be the greatest single source of error affecting accuracy and precision of calibration standards and quality control samples (QCs) in LC-MSMS assays. In this study, accumulated errors due to volume transfers during calibration and QCs preparation were investigated. The sample preparation design was based on the number of steps involved in a typical LC-MSMS assay. The weight of the volumes was used to calculate precision and errors. Each volume (in the range 0.1–9.7 mL) was weighed in replicates of 10. The precision of each step was measured and the overall precision calculated based on the work of (Meyer & Majors 2002). 5-Methoxytryptamine (5-MTT) and amitriptyline (ATT) were used as model compounds for LC-MSMS assay over a concentration range of 1–800 ng /mL (methods shown in Table 1). Deuterated internal standards were used to effectively eliminate errors due to injection volume and ion suppression. The chromatographic methods were developed to optimise peak shape and minimize integration errors. Cumulative sample preparation and measured precision values for QCs are shown in (Table 2). The performance of the amitriptyline assay was 99.50–101.96% accuracy and 0.38–1.60% precision. The performance of the 5-methoxytryptamine assay was 100.17–100.40% accuracy and 0.12–0.48% precision. We have concluded that the sample preparation is a significant source of error, contributing to the overall precision, except at the lower concentration where signal to noise effects have their greatest influence.

Table 1 LC-MSMS conditions for the analysis of ATT and 5-MTT

ATT		
Transitions monitored		(m/z 278→233) at 28 *eV* I.S ATT-d6 (m/z 284→233) at 28 *eV*
Mobile phase	A	Water/acetonitrile/formic acid (97/2.9/0.1; v/v/v)
	B	Acetonitrile/formic acid (99.9/0.1; v/v)
Elution		Gradient
Flow rate		1000 μL/min split at a ratio of 5:1 to the MS
Injection volume		10 μL
5-MTT		
Transitions monitored		(m/z 191→159) at 30 *eV* I.S 5-MTT-d4 (m/z 195→163) at 30 *eV*
Mobile phase	A	10 mM ammonium carbonate pH 10
	B	Acetonitrile
Elution		Isocratic
Flow rate		500 μL/min
Injection volume		10 μL

Table 2 Cumulative precision in preparing and measuring ATT and 5-MTT QCs

Concn (ng/mL)	Sample preparation precision (%)	Measured precision (%)	Total precision (%)
ATT			
3	0.9	1.60	1.86
30	0.86	0.67	1.12
300	0.81	0.38	0.93
600	0.72	0.45	0.85
5-MTT			
3	0.93	0.48	1.06
30	0.86	0.29	0.95
300	0.81	0.17	0.87
600	0.3	0.12	0.73

Meyer, V. R., Majors, R. E. (2002) *LC-GC Europe* **20**: 106–112

085

Temperature controlled Raman microscopy for the imaging of crystallisation and polymorphic transitions in frozen systems

L. J. Barrett[1], J. René Beattie[1], J. J. McGarvey[1,2] and V. Kett[1,3]

[1]Centre for Clinical Raman Microscopy, [2]School of Chemistry, [3]The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK. E-mail: v.kett@qub.ac.uk

Mannitol is extensively used as an excipient in freeze-dried pharmaceutical formulations since it promotes the formation of an elegant product. However, there are also problems associated with its use, including crystallisation upon reheating leading to vial cracking (Williams et al 1986) and reduction in its ability to act as a cryoprotectant (Izutsu et al 1993). Previous work (Kett et al 2003) using DSC, cold stage microscopy (CSM) and temperature controlled X-ray diffractometry (TXRD) has indicated that mannitol in frozen 3% w/w solutions crystallises into the β polymorph. In this investigation, this process has been probed using temperature controlled Raman microscopy (TRM), which enables the location of specific chemical functionalities within a 3D structure. The objectives were to locate the crystallising mannitol within the frozen solution and if possible to determine into which form(s) crystallisation is preferred. Mannitol (Roquette) was used as 3% w/w solutions in distilled water throughout the study. Samples were mounted in a Linkam TMS 600 biological cryostage with $N_{2(g)}$ purge and temperature controller and analysed using a Raman microscope (Horiba Jobin-Yvon LabRam HR800) equipped with an x-y motorised stage and an Olympus BX41 microscope ($\times$ 50 magnification was used, with an excitation wavelength of 514 nm). Raman data of single crystals of the α, β & γ polymorphs were acquired in each of the three possible orientations (x, y and z) with respect to the path and polarisation of the incident beam. The mannitol solution was cooled at 10°C min^{-1} to -30°C before annealing and data acquisition. Raman maps at the edges of the frozen annealed samples were recorded at 10-min intervals in the 2600–3600 cm^{-1} spectral region over an area of $12 + 28\,\mu m^2$ using a spacing of $3\,\mu$m. The spectral region for mapping was chosen to enable simultaneous monitoring of CH and OH vibrational modes of mannitol. The proportion of the spectrum arising from the different polymorphs and orientations was determined using linear combinations to fit the model spectra to the spectra at each point in the map. Significantly different spectra were obtained for each of the polymorphs and each of their orientations, allowing assignment of the polymorphic type and the orientation with respect to the incident laser pathway. The Raman maps obtained confirmed the presence of a boundary region that was rich in mannitol and also revealed that the mannitol concentration in this region increased with time. It was also demonstrated that, over time, this mannitol-rich region expanded inwards with the main icy body of the drop receding. Temperature controlled Raman microscopy has confirmed that the events observed using DSC correspond to crystallisation of the mannitol. TXRD had indicated that this was exclusively into the β form. However, Raman microscopy has shown that both the β- and α-forms are present. The technique has located, for the first time, the crystallising material within this sample. It clearly has potential for further use in following crystallisation processes in frozen pharmaceutical formulations.

Izutsu, K.-I. et al (1993) *Pharm. Res.* **10**: 1232–1237
Kett, V. L. et al (2003) *J. Pharm. Sci.* **92**: 1919–1929
Williams, N. A. et al (1986) *J. Parent. Sci. Technol.* **40**: 135–141

Poster Session 2 – Drug Delivery

086

Delivery of temozolomide hexyl ester prodrug through skin from VE TPGS microemulsion systems

P. Suppasansatorn[1,3], L. Du[2], B. R. Conway[1], Y. Wang[1] and U. Nimmannit[3]

[1]Aston Pharmacy School, Aston University, Birmingham B4 7ET, UK, [2]Chemical Drug Research Institute, Tasly Group, Tianjin City 300402, China and [3]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: y.f.wang@aston.ac.uk

In an attempt to develop a skin-deliverable congener for temozolomide (TMZ) to treat skin cancers, temozolomide hexyl ester (TMZ-HE) was identified as a potential prodrug because it is readily converted into parental temozolomide acid (TMZA) and has a promising permeability coefficient (K_p) and flux value (J) through rat and human skin (Wang et al 2002). TMZ-HE also demonstrated an equal cytotoxicity against the cancer cell lines as TMZ and TMZA, and significantly inhibited tumour growth in mice inoculated with melanoma via topical administration (Wang et al 2004). A formulation to deliver TMZ-HE through skin must demonstrate a high drug loading and stability of the system and the prodrug. Microemulsions (ME) were proposed as they are promising vehicles for skin delivery of drugs, demonstrating high drug loading capacity and a penetration enhancer effect (Kreilgaard 2002). As a starting point, we chose Vitamin E-TPGS (VE-TGPS) as a surfactant, oleic acid (OA) or isopropyl myristate (IPM) as an oil phase and isopropyl alcohol (IPA) as co-surfactant where appropriate. A gel VE-TPGS ME system was developed and a number of formulations were prepared (Table 1). The ME system and formulations were characterized using polarization microscopy and freeze fracture electron microscopy (FFEM). In vitro permeation of TMZ-HE from the ME formulations was studied using silicone membrane for 8 h and full-thickness hairless mice skin for 24 h with Franz diffusion cells. In the mouse skin permeation studies, TMZ-HE was extensively hydrolyzed by skin esterases to give TMZA (Wang et al 2002). The cumulative permeated TMZ-HE and TMZA were assayed using HPLC. The flux value (J) and permeability coefficient (K_p) were calculated. Skin stripping was used to determine TMZ-HE retention in the skin following skin permeation experiments. Drug retained in the skin were extracted and determined by HPLC. Compared with an aqueous control, VE-TPGS MS formulations increased the prodrug loading 35- to 75-fold and enhanced the permeation rate up to 7-fold through silicone membrane compared (Table 1). The fluxes and permeability coefficients of the prodrug from the OA ME and the IPM ME were significantly higher than from neat OA and IPM preparations. The OA ME showed more than 2-fold drug retention in stratum corneum (SC) than the IPM ME. In conclusion, VE-TPGS ME systems were showed to be a stable and effective vehicle resulting in high TMZ-HE loading and a permeation enhancing effect. The inclusion of OA is promising for a topical formulation, while IPM may promote transdermal absorption.

Table 1 Composition of the ME formulations, drug loading and drug flux through silicone membrane[a]

Formulation	Drug load (mg mL^{-1})	Flux (J) (nmol cm^{-2} h^{-1})	Ingredient (% w/w)[b]				
			W	OA	IPM	V	IPA
ME 1	15.3	67.64 ± 2.23	20	50	—	30	—
ME 2	20.8	79.01 ± 5.04	10	50	—	40	—
ME 3	31.8	101.00 ± 1.83	11	33	—	56	—
ME 4	22.4	162.86 ± 19.26	8	—	35	57	—
ME 5	21.6	263.58 ± 4.90	10	—	40	40	10
Control[c]	0.4	35.14 ± 5.95	—	—	—	—	—

[a]n = 3; mean ± s.d, [b]W = Water, V = VE-TPGS, [c]10% w/w propylene glycol in water.

Kreilgaard, M. (2002) *Adv. Drug Deliv. Rev.* **1**: S77–S98
Wang et al (2002) CN 02131346.6
Wang et al (2004) CN2004100686.80.7

087

Skin permeation studies of transdermal formulations of fluorescein through porcine ear stratum corneum

G. Oladiran and H. Batchelor

Medicines Research Unit, School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK. Email: oladirgs@aston.ac.uk

Pressure sensitive drug-in-glue transdermal formulations offer a simple means of preparing a formulation for application to the skin. This study compared the rate of drug release from three commercially available acrylic based glues and noted the effect of in vitro permeation with the addition of permeation enhancers. Disodium fluorescein was used as a model drug in all cases. The rate of drug release was measured from the patches into phosphate buffer solution (PBS) pH 7.4 over 72 h. The drug flux was calculated as the gradient of the mass released per cm^2 versus the time, this data is shown in Table 1. The results show that Durotak 87900A had the lowest rate of drug release and this glue was used in subsequent studies to note the effect of permeation enhancers on skin permeability. The effect of penetration enhancers, oleic acid and polyethylene glycol 400(PEG), singly or synergistically, on fluorescein permeation across full thickness dorsal porcine ear skin in static Franz diffusion cells was monitored. Porcine skin was excised from fresh carcasses and full thickness dorsal skin was dissected carefully with scalpel and scissors. PBS pH 7.4 was used as the receiver medium