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Short Communication

Fast atom bombardment mass spectrometry and the pharmaceutical analysis of corticosteroids*

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Introduction

The work described in this paper arose from attempts to study applications of fast atom bombardment (FAB) mass spectrometry to pharmaceutical analysis. In particular, steroids and other hormonal drugs present a continuing problem for regulatory and public analysts in analysing internationally available arthritis and other remedies. The numerous synthetic corticosteroids now available in a wide variety of chemical forms and formulations [1] require the development of analytical methods with high sensitivity, good reproducibility and a minimum of sample preparation. Although early reports on the LC–MS analysis of corticosteroids showed the potential of this method (despite an inherent thermal degradation problem [2]), the advent of fast atom bombardment mass spectrometry [3–5] clearly affords a breakthrough. Recent papers have shown that it is possible to obtain a resonable FAB spectrum from a mixture of steroid conjugates [6]. Although the fact that little or no fragmentation occurs [6, 7] facilitates the identification of individual components in complex mixtures, it is also synonymous with a lack of structural information. However, an offsetting advantage is that of accurately and efficiently obtaining the molecular weights of each constituent of a mixture of compounds without the necessity for prior separation. This study therefore investigates the behaviour of a series of corticosteroids when analysed under FAB conditions and evaluates the technique in terms of its usefulness for the detection and characterization of corticosteroids.

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Experimental

Fast atom bombardment mass spectra of 24 different corticosteroids were recorded in a glycerol and/or a thioglycerol matrix. All the compounds were USP reference standards. Samples of prednisone, cortisone acetate, prednisolone and dexamethasone were also extracted from commercial tablets for comparison purposes. All extractions were done according to the *British Pharmacopoeia* [8]. Spectra of nonextracted tablets were also recorded.

The spectra were recorded on a Finnigan MAT 312 spectrometer mounted with a saddle field atom gun (Ion Tech). The operating pressure was 5×10^{-5} mbar when the sample was run in glycerol and 2×10^{-4} mbar when run in thioglycerol. Xenon (research grade, 99.995%) was used for bombardment. The energy of the Xe beam was 8 kV, the accelerating voltage 3 kV, the multiplier voltage 2.2 kV, and the scanning speed was 10 s/scan. The mass range was *m/z* 100–1300.

Results and Discussion

All the spectra of the 24 corticosteroids used in this study exhibited pseudomolecular ions at $[M + H]^+$, and the majority of them also showed the formation of protonated dimers of the type $[2\,M + H]^+$. Spectra recorded in thioglycerol showed a net improvement in the intensity of the ions, compared with those recorded in glycerol. This improvement can be explained by the presence of the sulphur atom in thioglycerol, presumably because of the greater binding capability of the sulphur atom compared with that of oxygen [9]. A critical factor in FAB–MS is not the dissolution of the sample in the matrix solvent [10], but that the matrix can actually bind itself to the molecule under study [9].

Figure 1 shows a comparison of the positive ion FAB mass spectrum of triamcinolone acetonide recorded in thioglycerol and in glycerol. This is an example of a compound whose spectrum in glycerol shows mostly solvent peaks, whereas when recorded in thioglycerol it exhibits a strong pseudo-molecular ion, $[M + H]^+$, at *m/z* 435. Table 1 presents some selected FAB data for all the corticosteroids used in this study. The results obtained for the samples extracted from commercial drugs are also presented in Table 1. No difference is observed between the spectra of the authentic samples and those of the extracted materials, supporting previous claims that FAB can be used for identification purposes [6, 7, 10]. Spectra were also successfully obtained from unextracted tablets by powdering and mixing with the solvent.

The only apparent disadvantage in the FAB–MS of corticosteroids is the lack of fragmentation, as noted previously [6, 7]. However, this problem can be easily overcome in two ways. Firstly, even though the sample might be a complex mixture, the inherent lack of fragmentation of corticosteroids under FAB conditions allows for rapid molecular weight determination. It is then easy to resort to linked-scan techniques (B/E type) [11] under EI conditions as the fragmentation pathways of corticosteroids have been well characterized in these laboratories [12–14]. Secondly, linked-scan experiments can be performed under FAB conditions and, when induced by collision with a proper reagent gas, can yield more specific fragmentations characteristic of the substance under study (e.g. Table 2 and Fig. 2).

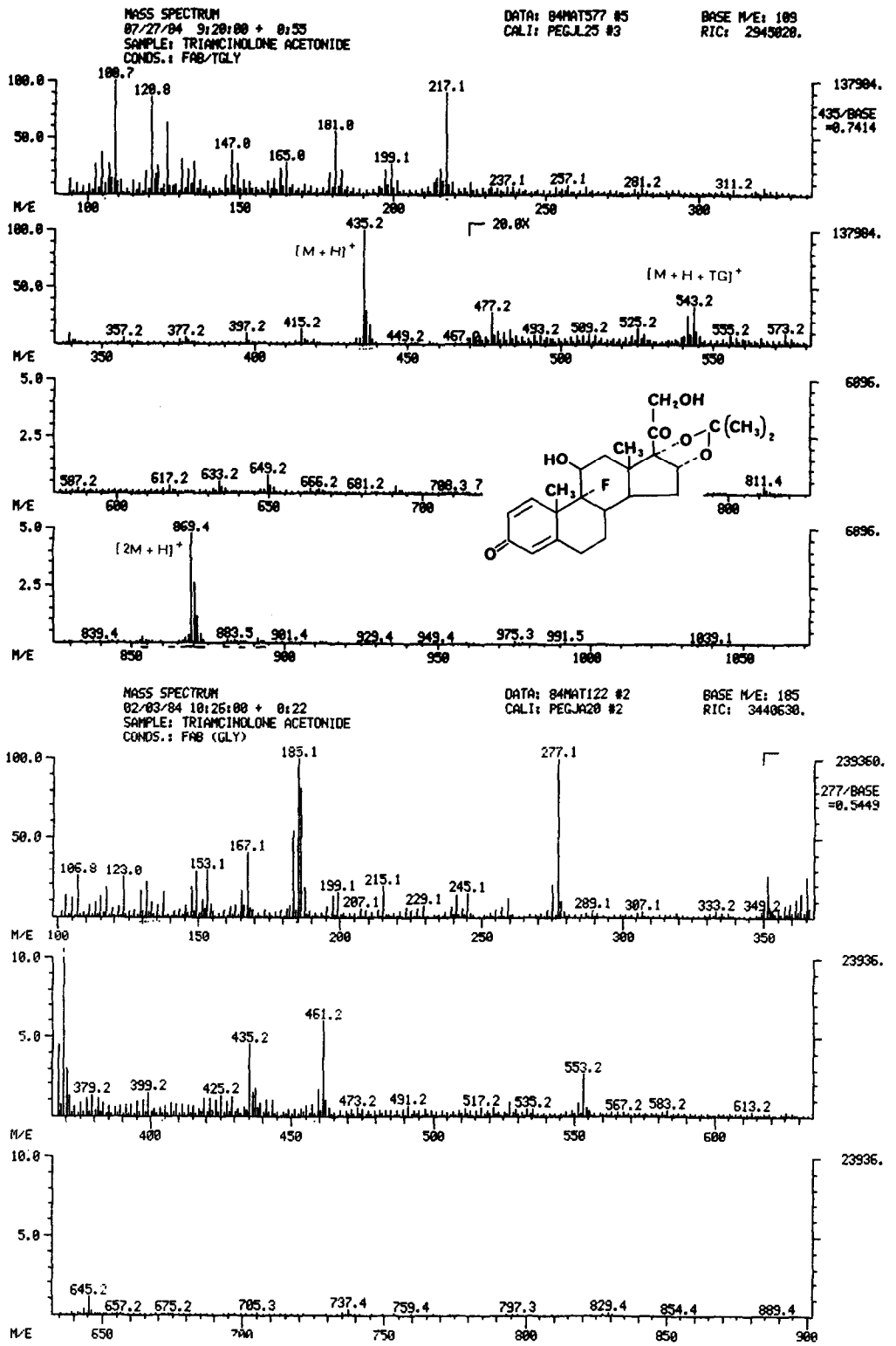

Figure 1
(a) FAB–MS spectrum of triamcinolone acetonide recorded in thioglycerol.
(b) FAB–MS spectrum of triamcinolone acetonide recorded in glycerol.

Table 1
Selected FAB–MS data for corticosteroids (corrected for matrix background)

Sample	$[M + H]^+$ *m/z*	% I (solvent) G	TG	$[2 M + H]^+$ *m/z*	% I (solvent) G	TG	Origin
Beclomethasone diproprionate	521	3	30	1041	NR	2.5	USP (1)
Betamethasone	393	41	*	785	NR	*	USP (1)
Betamethasone acetate	435	55	*	869	4.5	*	USP (1)
Betamethasone 17-valerate	477	11	40	953	NR	<1	USP (1)
Betamethasone 21-valerate	477	14	100	953	NR	12	USP (1)
Cortisone acetate	403	29	*	805	NR	*	USP (1)
Cortisone acetate	403	*	100	805	*	2	Tablet (2)
Cortisone acetate	403	*	100	805	*	3	Tablet (3)
Cortisone acetate	403	*	100	805	*	3	Tablet (4)
Desoxycorticosterone pivalate	415	NR	100	829	NR	2	USP (1)
Dexamethasone	393	44	*	785	1	*	USP (1)
Dexamethasone	393	*	70	785	*	4	Tablet (5)
Fludrocortisone acetate	423	43	*	845	NR	*	USP (1)
Flumethasone pivalate	495	9	33	989	NR	*	USP (1)
Fluocinonide	495	1	28	989	NR	<1	USP (1)
Fluocortolone hexanoate	475	15	100	949	NR	<1	USP (1)
Fluprednisolone	379	100	*	757	1	*	USP (1)
Hydrocortisone	363	100	*	725	1	*	USP (1)
Hydrocortisone acetate	405	5	24	809	NR	<1	USP (1)
Methylprednisolone	375	46	*	749	NR	*	USP (1)
Methylprednisolone acetate	417	30	*	833	NR	*	USP (1)
Paramethasone acetate	435	3	78	869	NR	3	USP (1)
Prednisolone	361	90	*	721	7.2	*	USP (1)
Prednisolone	361	*	100	721	*	6	Tablet (6)
Prednisolone acetate	403	6	40	805	NR	1	USP (1)
Prednisolone pivalate	445	8	15	889	NR	NR	USP (1)
Prednisone	359	80	*	717	2	*	USP (1)
Prednisone	359	*	100	717	*	6	Tablet (3)
Prednisone	359	*	100	717	*	4	Tablet (7)
Prednisone	359	*	100	717	*	6	Tablet (8)
Triamcinolone	395	100	*	789	2	*	USP (1)
Triamcinolone acetonide	435	11	100	869	NR	5	USP (1)

G = glycerol. TG = Thioglycerol. NR = not registered. No peaks were registered at those masses. * = the spectrum was not recorded in this solvent. 1 = Sample is a USP standard. 2, 3 . . . 8 = Manufacturer's reference number.

Table 2
Linked-scan (B/E) data for prednisolone obtained under FAB conditions from precursor ion 361^+

m_1	m_2	m*
361	343	325
	341	322
	325	292
	307	261
	283	221
	265	194
	239	158

Conditions: P = 5×10^{-5} mbar. Scan rate set at 11.

[+] Spectrum was CID using air as the collision (reagent) gas: P = 2×10^{-6} mbar (before addition of bombarding gas). Spectrum recorded in glycerol.

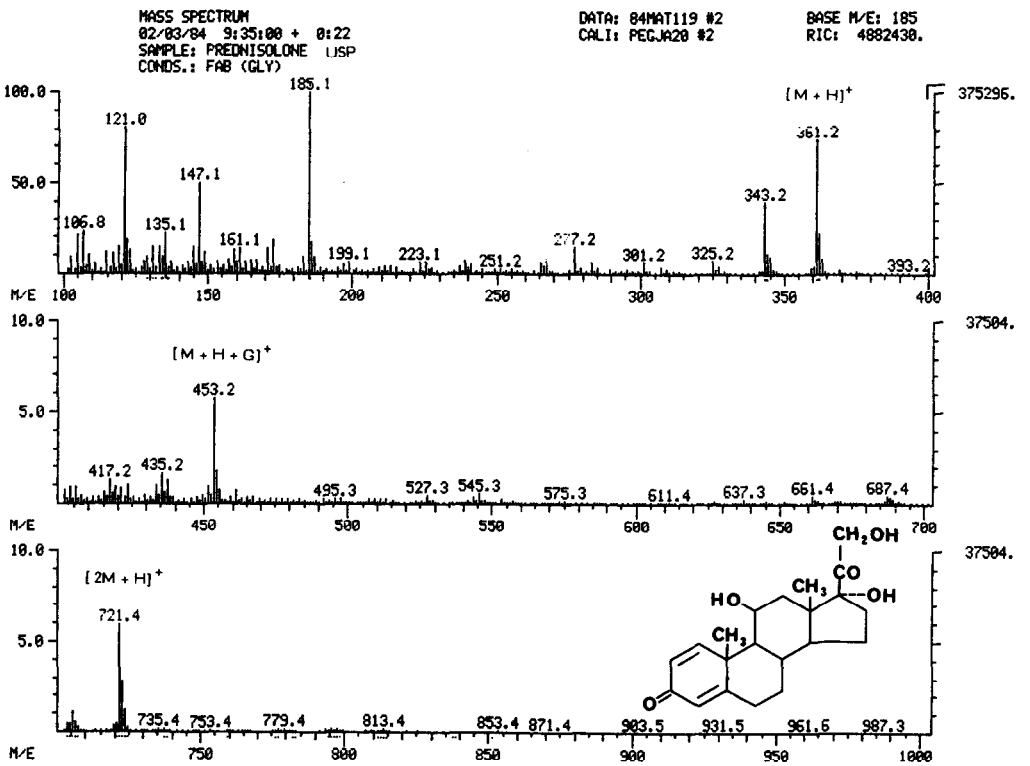

Figure 2
FAB–MS spectrum of prednisolone recorded in glycerol.

Conclusion

FAB–MS allows for the rapid and accurate determination of the molecular weight of corticosteroids. Better sensitivity is obtained when the spectra are recorded in thioglycerol; indeed, it is suggested that thioglycerol be the solvent of choice for corticosteroids. Even when samples are mixtures, it is possible, by the judicious combination of mass spectral techniques such as FAB followed by linked scan on conventional EI [12–14], to resolve and characterize fully the corticosteroids present in the sample.

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