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The physico-chemical properties of salmeterol and fluticasone propionate in different solvent environments

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Abstract

The physico-chemical properties of two anti-asthmatic drugs, salmeterol xinafoate and fluticasone propionate, have been studied in both aqueous and non-aqueous solvent environments. Ultraviolet-visible (UV-Vis) spectroscopy, fluorescence spectroscopy and electrospray ionisation mass spectrometry (ESI-MS) have been used to characterise the interaction of the drugs in 70:30 (v/v) methanol/water solutions. First derivative UV-Vis spectra measurements indicate that an interaction takes place between the two drugs in a binary solvent system. Fluorescence studies indicate that an increase in the concentration of fluticasone propionate results in a decrease in the fluorescence signal of the salmeterol for mixed solutions of the drugs. Analysis of a mixture of the two drug solutions using mass spectrometry also shows evidence of salmeterol-fluticasone propionate interaction and dimer formation with respect to both the salmeterol and the fluticasone propionate. Model metered dose inhalers (MDI) of both individual samples and mixtures of the drugs were formulated as suspensions in solvent CFC-113. The extent of deposition onto different inhaler components, such as the aluminium alloy canister, Teflon coated canister and the metering valve was evaluated by high-performance liquid chromatography (HPLC) of the methanol/water washings of the deposited drug(s). Changing the nature of the surface properties of the container resulted in a significant difference in the extent of deposition. The deposition of the individual drugs was found to increase as the dispersion concentration of the drug increases. However, the formulation based on a combination of the two drugs was found to show different deposition behaviour compared to the individual drug formulations. The deposition of the drugs, onto the aluminium alloy canister and the metering valve, decreases as the combined dispersion concentration of the two drug increases.

Keywords: Salmeterol xinafoate; Fluticasone propionate; Methanol/water; CFC-113; Deposition; UV-Vis; Fluorescence; ESI-MS; HPLC

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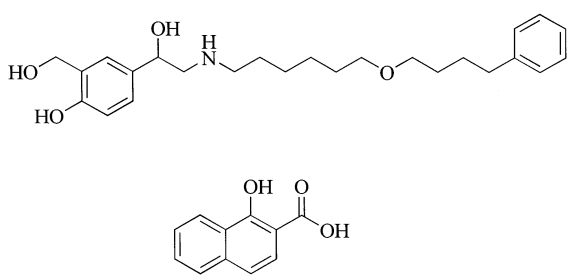
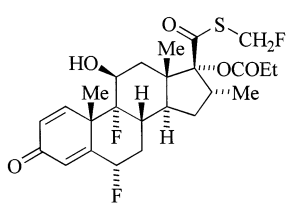
1. Introduction

Salmeterol xinafoate is a racemic form of the 1-hydroxy-2-naphthoic acid salt of salmeterol. It is the first of a new class of highly selective beta (2)-adrenoceptor agonists (Colthup et al., 1993; Johnson, 1995a; D'Alonzo and Tolep, 1997) having the desired pharmacological profile of a long acting bronchodilator. Salmeterol has a long carbon side-chain (Fig. 1a) which apparently, allows the molecule an extended duration of action-as long as 12 h or more (D'Alonzo and Tolep, 1997). Clinical studies (Dahl 1997; Weersink et al., 1997) have shown that salmeterol improves lung function and reduces airway hyper-responsiveness, persistent asthma symptoms and the need for rescue bronchodilators in patients receiving moderate-to high-dose inhaled corticosteroids. Fluticasone propionate [(*S*)-fluoromethyl-6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)-androsta-1,4-diene-17β-carbothioate] is a novel androstane glucocorticosteroid (Fuller et al., 1995) having potent anti-inflammatory activity (Fig. 1b). It has been shown to exhibit a higher degree of binding to human lung tissue compared with other currently available hydrophilic drug molecules such as budesonide, flunisolide and hydrocortisone which have been shown to improve therapeutic efficacy in the treatment of asthma (Fuller et al., 1995). A combination inhaler of salmeterol xinafoate and fluticasone propionate in a single MDI device has been used recently to improve the control treatment of asthma. The addition of salmeterol to the regimen is more effective than doubling the steroid dose (Dahl, 1997; Weersink et al., 1997) and further the same studies showed that after adding salmeterol to the regimen, the daily-inhaled corticosteroid doses can be reduced and oral corticosteroid courses are needed less frequently.



(a)



(b)

Fig. 1. Chemical structure of: (a) salmeterol xinafoate; and (b) fluticasone propionate.

Li et al. (1997) have reported a sensitive and selective high-performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry (HPLC/APCI/MS) method for the quantification of fluticasone propionate in human plasma. Measurements were made using an internal standard of (22R) budesonide. The technique offered the advantage of increasing the recovery of fluticasone propionate from blood plasma to 86.3% with a lower limit of detection for the drug being reported to be 0.2 ng/ml. Colthup et al. (1993) reported a sensitive, accurate, and precise method for the determination of salmeterol in plasma samples of animals for safety evaluation and pharmacokinetic studies. Samples were prepared by solid-phase extraction and quantitated by HPLC using fluorescence detection. Similarly, Chilton et al., (1995) have reported a clinical method for the determination of the 1-hydroxy-2-naphthoic acid salt of salmeterol in human plasma. The method involved solid-phase extraction using HPLC with fluorescence detection and the limit of detection was found to be 10 ng/ml. The majority of investigations reported for these drug substances have focused upon clinical pharmacology (Jack, 1991; Johnson, 1995b; D'Alonzo and Tolep, 1997; Austin et al., 1998) and method development techniques (Colthup et al., 1993; Chilton et al., 1995; Li et

al., 1997) for extending the detection limit of the drugs in human and animal plasma.

To date there has been very little fundamental work reported on the solution properties and/or deposition behaviour of these drugs in the scientific literature, particularly at concentrations typically found in MDI devices. We present, herein, what is believed to be the first study of the salmeterol xinafoate /fluticasone propionate system considering both the behaviour of the drugs in aqueous solution and as non-aqueous dispersions.

2. Materials and methods

2.1. Reagents

Salmeterol xinafoate and fluticasone propionate were kindly donated by Glaxo Wellcome Research and Development (Ware, UK) and used in micronised form (< 5 μm). HPLC grade methanol and acetonitrile, ammonium acetate and tetrabutylammonium hydrogen sulphate (TBAHS) were all purchased from Sigma–Aldrich (Gillingham, UK). Ninety nine percent weight per weight 1,1,2-trichloro trifluorethane (CFC-113) was obtained from The Basic Chemical Company (Bucks, UK). Water was purified in a Purite system (Oxon, UK).

2.2. UV-Vis spectroscopy

A UVIKON type 860 ultraviolet-visible spectrometer (Kontron Instruments, Zurich, Switzerland) with matched 1 cm quartz cells was used in all the UV experiments. Analysis were performed using both direct and first-order derivative modes over a wavelength range of 200–400 nm. Standard solutions of 0.24 mM of salmeterol xinafoate and fluticasone propionate in 70:30 v/v methanol/water proportions were prepared individually. Different mixed standard solution of salmeterol xinafoate and fluticasone propionate was scanned against the same concentration of individual drug solutions placed in the reference cell. The spectra were then compared with the spectra of individual drug samples at the same concentration scanned against methanol/water.

2.3. Fluorescence spectroscopy

For the fluorescence experiments a Perkin–Elmer fluorescence spectrophotometer (Model-MPF-43, Connecticut, USA) was used. The fluorescence detection of salmeterol[1] was performed by excitation at 344 nm and measuring the emission at 411 nm. The fluticasone propionate may be excited at 370 nm, however it shows no fluorescence activity at 344 nm, the wavelength at which all fluorescence experiments were carried out. The fluticasone propionate is therefore to all intent and purposes 'invisible' at the fluorescence excitation wavelength used.

The fluorescent properties of salmeterol in the presence of various fluticasone propionate concentrations were also examined. The relationship between fluorescence intensity and analyte concentration is:

$$F = k\mathrm{QE}P_0(1 - 10^{[-\varepsilon bc]})$$

where F is the measured fluorescence intensity, k is a geometric instrumental factor, QE is the quantum efficiency (photons emitted/photons absorbed), P_0 is the radiant power of the excitation source, ε is the wavelength-dependent molar absorptivity coefficient, b is the path length, and c the analyte concentration. Expanding the above equation in a series and dropping higher terms gives:

$$F = k\mathrm{QE}P_0(2.303\varepsilon bc)$$

This relationship shows that fluorescence intensity is linearly proportional to analytical concentration.

Stock solutions of salmeterol xinafoate and fluticasone propionate of 0.24 mM were prepared separately. Solutions of different mole fractions of the drugs, ranging from 0.0 to 1.0 with respect to the drug concentration, were prepared to give a final volume of 10 ml at a fixed and constant concentration of 0.024 mM. The fluorescence intensities of all the mixed drug samples were recorded at room temperature against solutions (at the same concentration as the mixture in each

[1] *Note*: A factor of 0.6883 was used to convert salmeterol xinafoate (salt) to salmeterol (base).

case) of salmeterol xinafoate without any fluticasone propionate. The fluorescence intensity of salmeterol in the different experiments was plotted against mole fraction of salmeterol xinafoate.

2.4. ESI-MS

Mass spectra were obtained using an Applied Biosystems 140B modular system consisting of a high pressure binary syringe pump, Applied Biosystems 112A modular oven/injector (Perkin–Elmer, Warrington, Cheshire, UK) and a Micromass Platform I (Wythenshawe, Manchester, UK) mass spectrometer equipped with a standard electrospray ionisation (ESI) source. The interface settings were source temperature 40°C, capillary 3.6 kV and cone volts 25 V. The drying gas was maintained at ca. 300 l/h and nebulizer gas at ca. 20 l/h, respectively. Data were acquired in positive ionisation full scan mode over the range 0–1200 amu. Dwell times of 1 s were used to monitor each ion. The data were collected and analysed using a Mass Lynx V 2.22 system.

For experimental investigations of the interaction between the two drugs, a series of different drug compositions, in 70:30 v/v methanol/water, were prepared by fixing the salmeterol xinafoate concentrations at 25 μM while the fluticasone propionate concentration was varied from 0–80 μM. Approximately 50 μl of each mixed solution was introduced every 4 min into the ESI-MS interface by loop injection using a flow rate of 50 μl/min. Selected ion recording (SIR) was performed to monitor the values of intensities of m/z ions at: 416.5 $(\text{salmeterol} + \text{H})^+$; 523.4 $(\text{fluticasone propionate} + \text{Na})^+$; 916.4 $(\text{salmeterol-fluticasone propionate} + \text{H})^+$; and 1023.3 $(2 \times \text{fluticasone propionate} + \text{Na})^+$. The experiment was repeated keeping the fluticasone propionate concentration constant and varying the salmeterol xinafoate. The peak area of each ion monitored was recorded for each compound mixture that produced a measurable response in positive ESI. Chemical noise was compared by infusing the test eluents into the mass spectrometer at a flow rate of 50 μl/min.

2.5. HPLC

The HPLC system consisted of a model 6000A solvent delivery system (Waters Associates, Milford, MA, USA). Separation was achieved using a Hypersil BDS C18 column (5 μm, 200 × 4.6 mm id). The column temperature was maintained at 40°C using a model 7716 HPLC column block heater (Jones Chromatography). The mobile phase was a mixture of methanol-acetonitrile-deionised water (30:30:40 v/v) which also contained 0.6% w/v ammonium acetate and 0.2% w/v tetrabutylammonium hydrogen sulphate (TBAHS). Before use the solvent was filtered through a 0.45 μm filter (Millipore, Watford, UK). The analysis was carried out at a flow rate of 1.5 ml/min. The injection volume was 20 μl and detection was by UV (LDC/Milton Roy, Des Plaines, USA) at 228 nm. The sensitivity of the detector was kept fixed throughout the experiment. Data handling was carried out using a C-R3A Chromatopac (Shimadzu, Kyoto, Japan) system.

2.6. Standard preparation

Mixed standard solutions consisting of 24 μM salmeterol xinafoate and 100 μM fluticasone propionate were prepared. Typically, 1.5 mg of salmeterol xinafoate analytical working standard and 5.0 mg of fluticasone propionate analytical working standard were weighed into a 100 ml volumetric flask. Thirty millilitres of deionised water and 70 ml of HPLC grade methanol were added separately to the mixed solution. Each sample was sonicated for 10 min to dissolve the solutes. The solutions were made up to the final volume with HPLC grade methanol and mixed well.

2.7. Deposition studies

Known weights of salmeterol xinafoate were transferred into an aluminium alloy canister. The unit was then filled with a known weight of solvent CFC-113. A number of serial suspensions were made in triplicate to check the repro-

ducibility of the method. The suspensions were then crimped with the metering valve (Valois DF60) to prevent evaporation of the solvent. Each suspension was dispersed by sonication for 10 min to remove any entrapped air and to promote dispersion. The filled MDI units were shaken horizontally for a minimum of 8 h to allow deposition of the drug onto the canister and the valve prior to testing. The can was continuously agitated so that none of the suspension was allowed to separate during this period. The metering valve was then cut open and the suspension discharged from the canister. The solvent was left to evaporate, leaving the powder deposited on the interior of the canister. The aluminium alloy canister and the metering valve were washed separately with HPLC grade methanol and the washings transferred quantitatively into 50-ml volumetric flasks, containing 15 ml of deionised water. This procedure was repeated to wash all the deposited layer of the powder. The flask was shaken to ensure good mixing and was made up to the final volume with methanol. The drug deposited on to the surfaces of MDI was measured using HPLC. Usually, 50 μl were injected via an injection port with 20.0 μl injection loop. Each sample and analytical working standard was injected at least twice to guarantee the reproducibility of the results. The foregoing procedure was repeated for fluticasone propionate and mixed dispersions of salmeterol xinafoate /fluticasone propionate as the depositing agent. The drug assays were controlled by running the analytical working standards at two concentrations assayed in duplicate during each analysis. The solution concentration of the individual drug samples were then quantified from the peak area produced by the working standards. Calibration of standards over the concentration range of 1.0–10.0 μg/ml ($n = 6$) of the salmeterol and fluticasone propionate were prepared by mixing the drugs in methanol/water. The correlation coefficient of the linear regression line for both salmeterol and fluticasone propionate were found to be 0.999.

3. Results and discussions

Individual drug solutions were first analysed by scanning in the UV-Vis region over the 200–400 nm wavelength range against a reference solution of methanol/water. The maximum absorbance for fluticasone propionate was found at 237 nm and for salmeterol at 216 and 251 nm. Individual drug solutions at the same concentration as in the mixed drug solution were also used in the reference cell to eliminate background interference. Fig. 2 shows the overlay UV spectra of the individual drugs with the spectra of the drugs subtracted from the mixed solution. It can be seen from the curves that there is a significant difference between the spectra as neither of the maximum absorbance of the subtracted spectra from the mixture compounds gave the same absorbance reading when they were compared on the same scale to the individual drug compounds. These data suggest that each drug affects the light absorption characteristics of the other. Fig. 3a shows the first derivative curves for 8 μM fluticasone propionate solution measured against methanol/water and Fig. 3b shows the spectrum for mixed drug solution of 8 μM fluticasone propionate and 32 μM salmeterol xinafoate scanned against 32 μM salmeterol xinafoate in the reference cell. It can be seen from the spectrum that a shoulder appears at 265 nm in the solution mixture. The appearance of the peak suggests that an interaction between the two drugs is taking place in solution.

The fluorescence behaviour of the salmeterol in the presence of fluticasone propionate was estimated by the wavelength dependence of 344 nm excitation and emission at 411 nm. Fig. 4 shows the fluorescence intensity of salmeterol plotted against the mole fraction of salmeterol xinafoate. The correlation coefficient of a linear regression of 0.99 were observed for both salmeterol and mixed solution. It can be seen that the fluorescence intensity of the standard salmeterol is different as compared to the same concentration of salmeterol in a mixture solution. The change in fluorescence intensity further indicates that an interaction of the drugs is taking place in a methanol/water solvent.

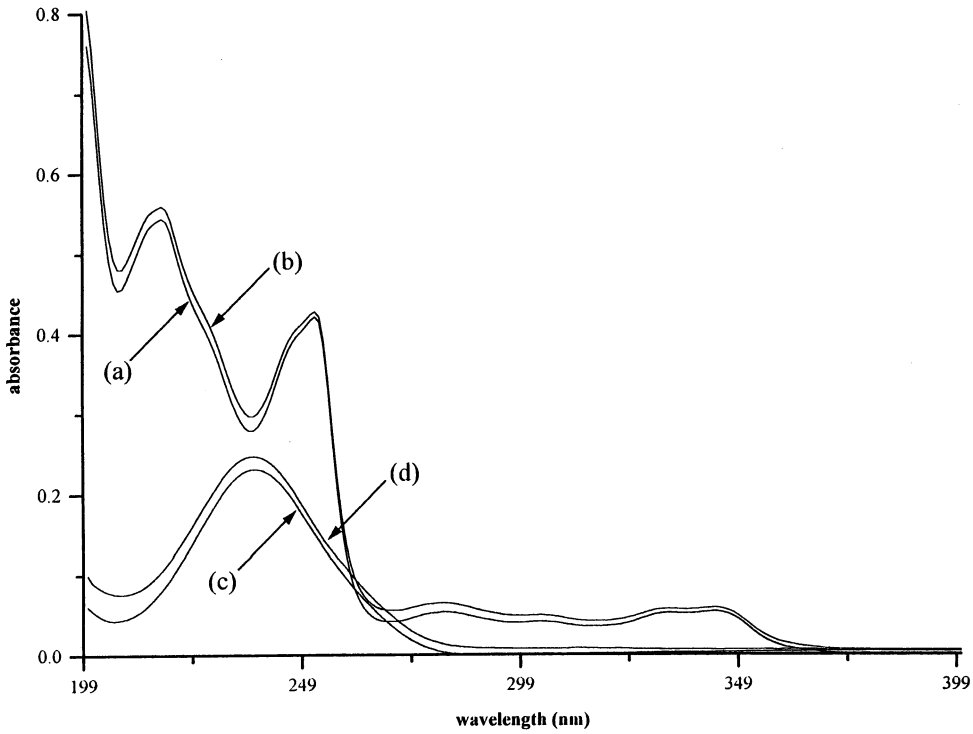


Fig. 2. UV-Vis spectrum of: (a) 12 μM salmeterol against methanol/water; (b) a mixture of 12 μM salmeterol and 12 μM fluticasone propionate measured against a reference of 12 μM fluticasone propionate; (c) 12 μM fluticasone propionate against methanol/water; and (d) a mixture of 12 μM salmeterol and 12 μM fluticasone propionate measured against a reference of 12 μM salmeterol.

Fig. 5 shows the positive ion electrospray spectra of fluticasone propionate, salmeterol and the mixed drug solution in methanol/water collected using the same parameters described in the experimental mass spectrometry section. The mass spectrum for fluticasone propionate shows an ion at 501.3 corresponding to $(M+H)^+$ and that at 1001.1 to $(M_2+H)^+$ ions. For salmeterol a peak at 416.4 corresponds to $(M+H)^+$ ion with a small peak at 831.5 which represents a signal for the $(M_2+H)^+$ ion. All the above peaks, with m/z values of 416.4, 501.3, 831.5 and 1001.2, were observed in the mixed solution spectrum indicating the presence of monomeric and dimeric species in the drug solutions. A number of monomeric and dimeric species were also observed by Mistry et al. (1997, 1999) using fluticasone propionate dissolved in acetonitrile/water mixture by NMR and HPLC-NMR/MS. The peak length of SIR of m/z ions at 416.5, 523.4, 916.4 and 1023.2 were measured for mixed drug solutions with increasing concentration of fluticasone propionate. The SIR response was show to increase as the concentration of fluticasone propionate increased. The foregoing results show that the mixed drug solution contain various compositions including monomers and dimers; and that an intermolecular association occurs between salmeterol and fluticasone propionate.

An example of a typical chromatogram of a standard sample of salmeterol and fluticasone propionate is shown in Fig. 6. The retention time of salmeterol and fluticasone propionate was consistent during analytical HPLC runs and the drugs eluted at 3.6 and 9.2 min, respectively. Plots of amount of drug deposited on to different MDI surfaces as a function of salmeterol and fluticasone propionate concentration are presented in Fig. 7a and Fig. 7b. The data in Fig. 7a and Fig. 7b clearly show that the deposition of the drugs

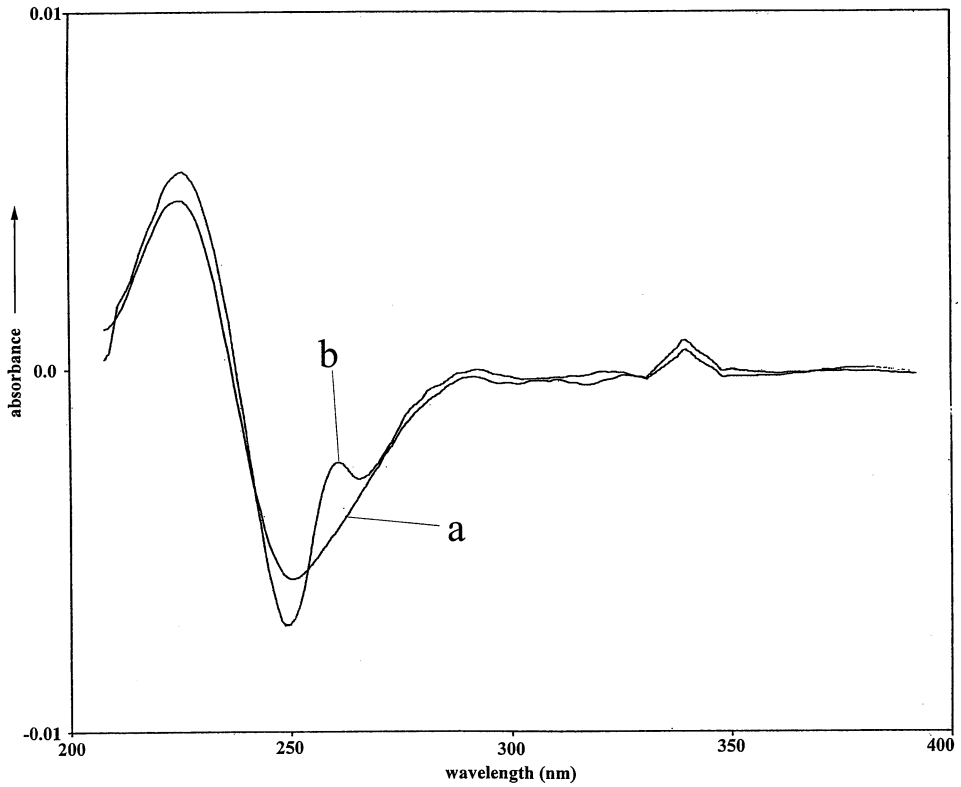


Fig. 3. First-order derivative spectra of: (a) 8 μM fluticasone propionate against methanol/water; and (b) a mixture of 32 μM salmeterol and 8 μM fluticasone propionate measured against a reference of 32 μM salmeterol.

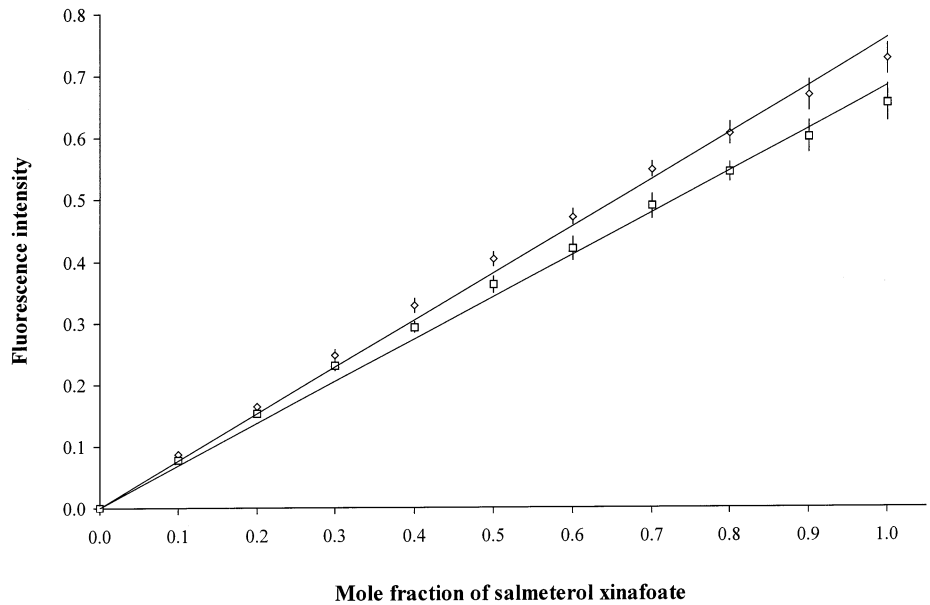


Fig. 4. Fluorescence intensity for salmeterol as a function of the mole fraction of salmeterol. (◇) Salmeterol only and (□) salmeterol/fluticasone propionate in 70:30 v/v methanol/water solvent.

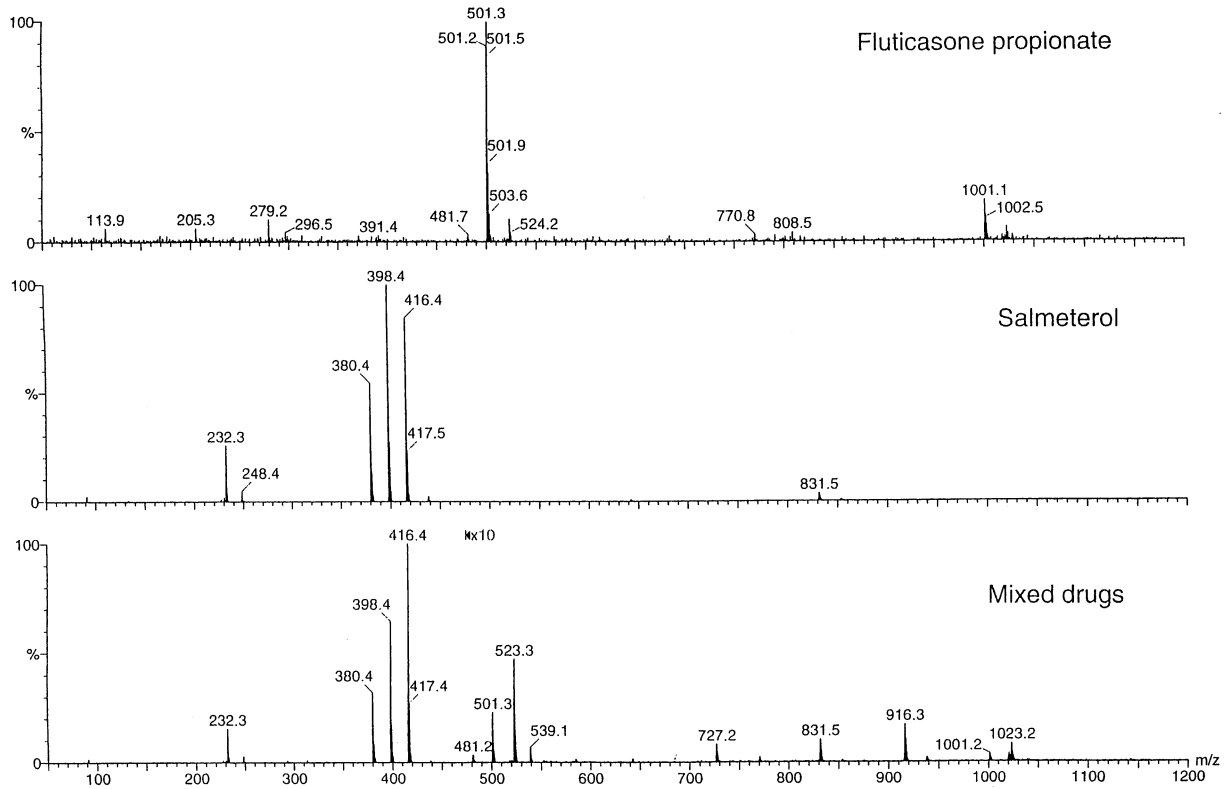


Fig. 5. Positive ion electrospray mass spectra of salmeterol, fluticasone propionate and a mixture of two compounds.

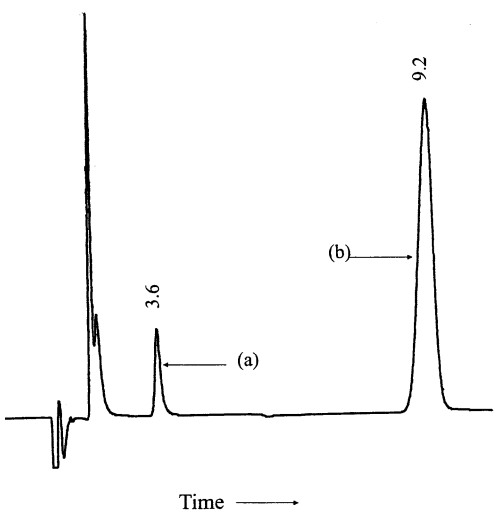


Fig. 6. Typical HPLC chromatogram of a mixed solution of salmeterol and fluticasone propionate in 70:30 v/v methanol/water solvent. Peak (a) corresponds to salmeterol and peak (b) corresponds to fluticasone propionate.

on to the surface increases with increasing drug concentration. This increase reaches its maximum plateau as the number of available sites on the surface reduces. The data also show that the weight of individual drug deposited on to the aluminium alloy, Teflon coated canister and metering valve were significantly different. The nature of the surface is clearly an important factor in drug deposition. When the aluminium canister is coated with Teflon the amount of drug deposited was found to change significantly, especially with fluticasone propionate (Fig. 7b). The amount of deposited drug is significantly lower for fluticasone propionate onto the coated canister compared to the uncoated aluminium canister. Coating the canister changes the nature of the aluminium alloy surface and leads to a reduction in deposition of the drugs.

The deposition of the drugs from the mixed dispersion of salmeterol xinafoate and fluticasone propionate was also analysed using HPLC. Fig.

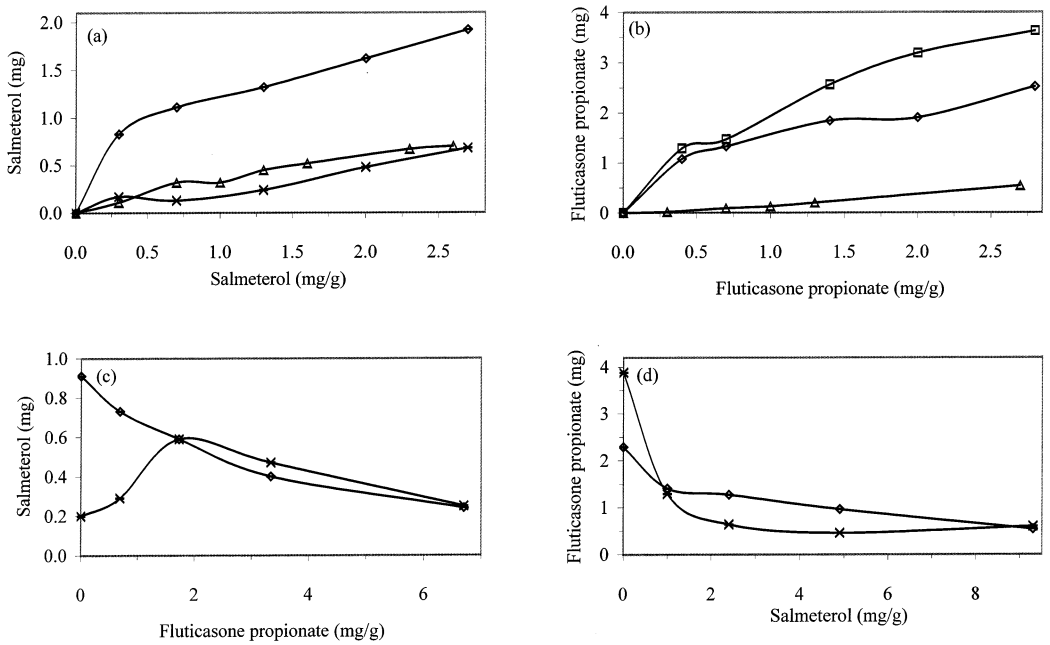


Fig. 7. The weight of drug depositing on to the MDI surfaces as a function of drug concentration. (a) Deposition of salmeterol with increasing salmeterol concentration; (b) deposition of fluticasone propionate with increasing fluticasone propionate concentration; (c) deposition of salmeterol from mixed dispersion with increasing fluticasone propionate concentration; (d) deposition of fluticasone propionate from mixed dispersion with increasing salmeterol concentration. The surfaces in each experiment are as follows (×) aluminium alloy canister; (◇) metered valve; (△) Teflon coated canister.

7c and Fig. 7d show the deposition of mixed drugs with increasing concentration of fluticasone propionate and salmeterol xinafoate concentration, respectively. This indicates the deposition behaviour of the mixed dispersion is different from the deposition of the individual drugs. The mixed dispersion shows less tendency towards deposition on to the MDI surfaces, i.e. aluminium alloy canister and plastic metering valve; in comparison to the individual suspensions. At higher concentrations of fluticasone propionate in the mixed dispersion, less salmeterol xinafoate is deposited (Fig. 7c). The dramatic change could be due to an improvement in drug-solvent interaction at higher fluticasone propionate concentration which leads to minimal surface deposition of the salmeterol xinafoate. This demonstrates that both the two drug particles interact and tend to have a relatively high affinity toward the CFC-113 than to either the canister or the metering valve. A similar phenomenon was observed for fluticasone propionate in the mixed dispersion.

The balance of interactions in CFC-113 is clearly delicate and of considerable importance with respect to drug deposition. For drugs by themselves the nature of the surface is a dominant factor, however in mixed dispersions the drug–drug and resultant solvent interaction appears to be of greater importance.

4. Conclusions

This study has shown that in a 'better' solvent environment of methanol/water an interaction takes place between the two drugs, salmeterol and fluticasone propionate, with clear evidence of dimer formation (i.e. salmeterol/salmeterol and fluticasone propionate/fluticasone propionate). It is likely, therefore, that similar behaviour will take place in a much 'poorer' solvent such as

CFC-113 where the drug–solvent interaction is less favourable than in a methanol/water mixture. It is interesting that such interactions facilitate less deposition of the drugs with the complexes formed favouring the solvent environment compared to the single drug species.

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