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# Determination of surface-adsorbed excipients of various types on drug particles prepared by antisolvent precipitation using HPLC with evaporative light scattering detection

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**Abstract**

A common challenge in the development of new drug substances is poor dissolution characteristics related to low aqueous solubility. One approach to overcome this problem is antisolvent precipitation in the presence of polymers or surfactants, which may enhance the dissolution rate through reduced particle size and increased wettability. In this study, a simple method based on size exclusion chromatography (SEC) with evaporative light scattering detection (ELSD) was developed for the determination of polymers and surfactants adsorbed to drug particles prepared by antisolvent precipitation of the poorly water-soluble model drug Lu 28–179. Detection of many polymeric excipients and surfactants is problematic due to the lack of UV-absorbing chromophores, but ELSD proved successful for the direct determination of the investigated compounds. A mixed mode column was used to effectively separate each of the excipient structures from the drug. The mobile phase comprised acetonitrile–ammonium formate (20 mM; pH 6.5) (50:50, v/v) at a flow-rate of 0.6 ml/min. Qualification studies showed that the method was adequately sensitive and precise with limits of detection between 0.72 and 4.32 μg/ml. Linearity of the calibration curves was achieved by log–log modelling. The method was applied for determination of nine polymeric excipients and surfactants adsorbed to particles of the model drug. The extent of excipient adsorption varied between 0.07 and 1.39% (w/w) of the total particle weight.


*Keywords:* Poorly soluble drugs; Antisolvent precipitation; Surfactants; Polymers; Size exclusion chromatography (SEC); Evaporative light scattering detection (ELSD)

## 1. Introduction

An increasing number of newly discovered drug substances suffer from poor aqueous solubility, which is associated with poor dissolution characteristics. Dissolution rate in the gastrointestinal tract is the rate-limiting factor for the absorption of many of these drugs, which therefore suffer from poor oral bioavailability [1,2]. One approach to surmount this problem is to produce particles with improved surface characteristics by precipitation of poorly soluble drugs in the presence of excipients [3,4]. During the precipitation process, both polymeric and surface active excipients may adsorb to the particle surface, providing a hydrophilic coating of the particles, which may increase the dissolution rate through an increase in wettability. Further, the presence of polymers reduces the particle size of the formed particles through steric stabilization of the formed suspension, leading to an increase in surface area and thus area available for dissolution [5,6].

The drug substance Lu 28–179 (Fig. 1) is a small organic molecule, which is poorly water-soluble. As part of an ongoing study to improve the dissolution rate of Lu 28–179, microparticles of the drug have been prepared by antisolvent precipitation in the presence of nine different excipients (Fig. 1). During each precipitation experiment, one excipient was present. This resulted in nine batches of microparticles of Lu 28–179, surface-coated with different excipients. The excipients varied in size

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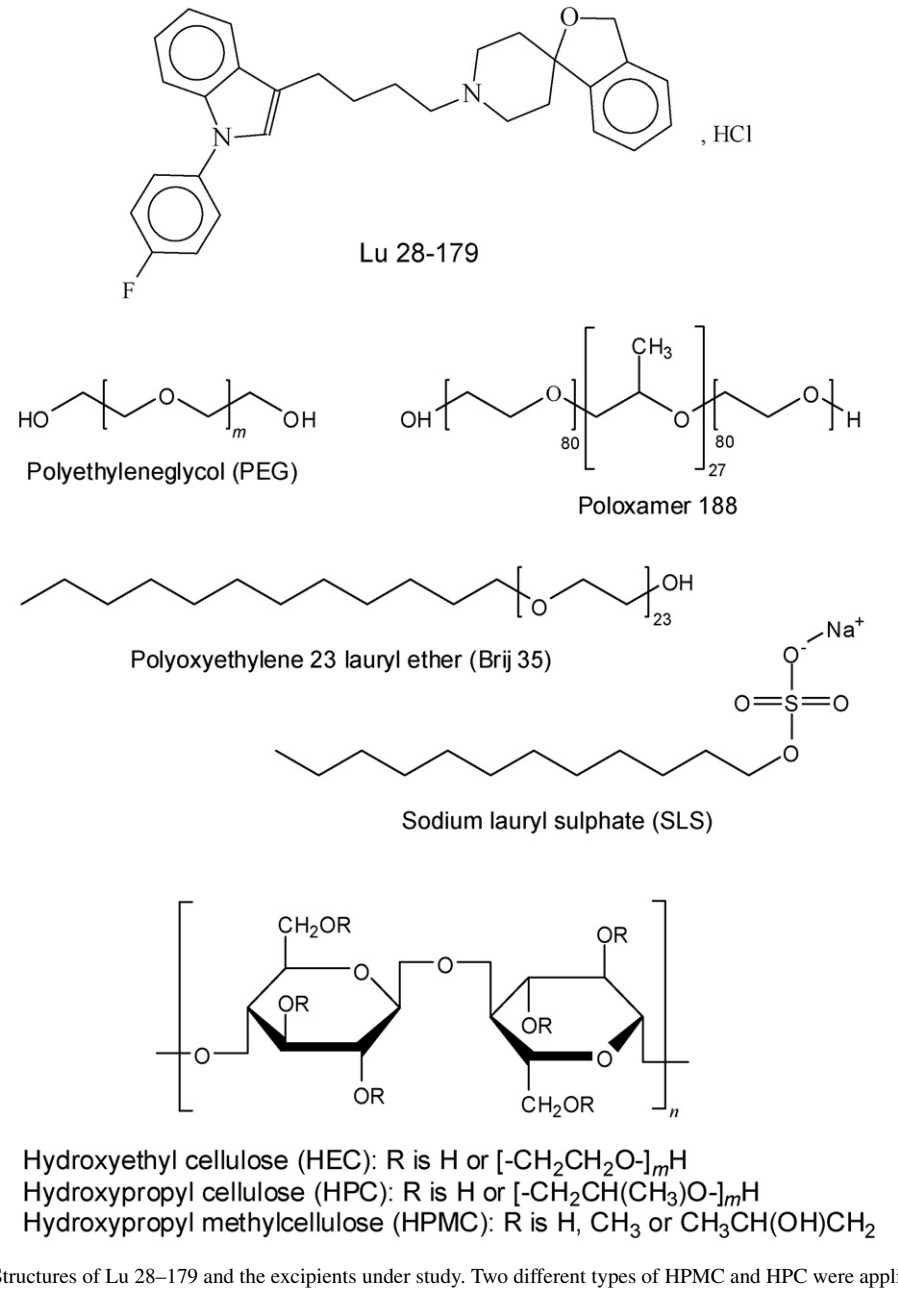


Fig. 1. Structures of Lu 28–179 and the excipients under study. Two different types of HPMC and HPC were applied.

and structure ranging from the low molecular weight sodium lauryl sulphate (SLS) to large polymers, such as hydroxypropyl methylcellulose (HPMC).

In order to study interactions between drug substances and excipients during precipitation, a quantitative determination of excipient adsorption to the drug particles is important, but presents several challenges. First of all, the diversity between relevant excipients is extreme with regard to both size and structure. They must be separated from the poorly soluble drug substance, which most often is a small organic molecule. Size exclusion chromatography is commonly used for the separation and quantification of polymers [7]. The separation is based on the hydrodynamic volume of the molecules, which depends on the molar mass, the solvent and the chemical composition of the polymer [8]. Hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC) in pharmaceutical formulations have been determined [7,9], and poly(lactic-co-glycolic acid) (PLGA) degradation in microparticles was followed using SEC with refractive index detection (RI) [10,11]. SEC has also been used for molar mass determinations of Poloxamer [8]. In contrast, lower molecular weight surfactants are commonly separated by normal or reversed phase chromatography [12,13], which is also often the choice for small organic drug substances [14]. Here the mechanism of separation is partitioning. Finding one separation system applicable to analysis of a wide range of excipients relevant for surface optimization of drug particles through antisolvent precipitation was one aim of the present study.

Detection methods alternative to UV constitute other challenges due to the lack of chromophores of many polymers and

surfactants [7,15]. Mass spectrometers (MS), refractive index detectors (RI) and evaporative light scattering detectors (ELSD) can all be coupled to an HPLC and have all been used for the determination of various non-UV absorbing compounds [15–17]. MS is more sensitive than both RI and ELSD, but compared to MS, ELSD is less expensive and easier to operate [15,18]. Studies comparing RI and ELSD have proven ELSD to be superior in terms of sensitivity and precision [7,17].

Recently, the use of ELSD has increased considerably due to its ability to detect non-volatile compounds regardless of structural characteristics [7,9]. In the pharmaceutical industry, it is used for the determination of drugs, impurities, raw materials and excipients [16,19–21]. In principle, ELSD is based on nebulisation of the eluent to droplets, evaporation of the mobile phase and detection of the light scattered by the remaining particles [7,18,19].

A few methods for the determination of polymers or surfactants using HPLC–ELSD are reported. Hydroxypropyl methylcellulose (HPMC) adsorbed to particles of ibuprofen produced by antisolvent precipitation has been determined using HPLC–ELSD [9], and SEC with ELSD and RI detection has been evaluated for the determination of hydroxypropyl cellulose (HPC) bound to drug particles in colloidal dispersions [7]. Methods for Poloxamer 188 and various types of polyethylene glycols (PEG) using SEC or HPLC with MS or ELSD have been reported [15,17,22,23], and sodium lauryl sulphate (SLS) and Brij 35 have been determined for non-pharmaceutical purposes [12,24]. However, no method suitable for determination of all of these different excipients has been reported.

The aim of the present work was to develop a method suitable for the quantitative determination of nine different excipients present on the surface of microparticles prepared by antisolvent precipitation of the poorly water-soluble drug Lu 28–179. The method was based on size exclusion chromatography with evaporative light scattering detection.

## 2. Experimental

### 2.1. Chemicals and reagents

The active pharmaceutical ingredient was the hydrochloride salt of the compound 1′-[4-[1-(4-fluorophenyl)-1-H-indol-3-yl]-1-butyl]spiro[*iso*-benzofuran-1(3H), 4′ piperidine] (Lu 28–179, molecular weight 491.06 g/mol, solubility of the hydrochloride salt in water 150 μg/ml, $pK_a \sim 9$, $\log P \sim 8.5$). The drug was supplied by H. Lundbeck A/S, Denmark. The excipients were hydroxypropyl methylcellulose (HPMC; Metolose® 90 SH 4000 SR and Metolose® 90 SH 100,000 SR, Shin Etsu, Japan), hydroxyethyl celloluse (HEC; Natrosol® Pharm G, Aqualon, France), hydroxypropyl cellulose (HPC; Klucel® LF Pharm and Klucel® MF Pharm, Aqualon, France), Poloxamer 188 (Lutrol® F68, BASF, Germany), Polyethyleneglycol (PEG; Macrogolum 6000, Unikem, Denmark) sodium lauryl sulphate (SLS; Unikem, Denmark), polyoxyethylene 23 lauryl ether (Brij 35, Sigma Chemical Co., USA). Two types of the polymers HPMC and HPC were applied; HPMC 4000 and 100,000 cP (viscosities of the polymers in 2% (w/w) aqueos solution) and HPC molecular weight 95,000 and 850,000. HPLC grade acetonitrile and ammonium formate were obtained from Sigma–Aldrich (Germany), and deionized reagent water was prepared by a water purification system (Holm & Halby, Denmark).

### 2.2. Preparation of microparticles by antisolvent precipitation

Microparticles of Lu 28–179 were produced by antisolvent precipitation in the presence of excipients. Nine batches were produced, each containing Lu 28–179 and an excipient. First, an organic solution of the drug was prepared by dissolving 500 mg of the drug in 50 ml of ethanol. Then 200 ml of an aqueous solution containing a polymer or a surfactant (0.025%, w/v) was added rapidly under stirring conditions to the drug solution. This caused super saturation with respect to the drug and subsequent nucleation and particle growth. After 60 min, the particles had reached their final particle size distribution (measured by laser diffraction), which was governed by the excipient present. The particles were isolated by vacuum filtration followed by three consecutive washings with 10 ml of cold water to remove any non-adsorbed excipient. Following isolation, the particles were dried over anhydrous silica in a desiccator.

### 2.3. Chromatographic conditions

The HPLC system comprised an isocratic pump model L-6200, an autosampler (AS 4000A), a column thermostat (L-5025) and a D-6000 Interface, all obtained from Merck Hitachi, Japan. Data acquisition and analysis were performed using D-7000 HSM software, also obtained from Merck Hitachi. The analytical column was a Shodex Asahipak GS-320HQ mixed mode column (300 mm × 7.5 mm i.d.), 6 μm particle size. Column temperature was maintained at 30 °C. The mobile phase consisted of acetonitrile–ammonium formate (20 mM; pH 6.5) (50:50, v/v), which was degassed by sonication for 15 min prior to use. A flow-rate of 0.6 ml/min was used, and run time was set to 30 min. The injection volume was 100 μl. The evaporative light scattering detector was a model PL-ELS 2100 from Polymer Laboratories, USA. Nitrogen was used as nebulization gas at a gas flow of 1.6 l/min. A nebulizer temperature of 50 °C and an evaporation temperature of 90 °C were applied.

### 2.4. Sample preparation

During preliminary experiments, it became clear that the amount of excipient adsorbed on the drug particles was less than 1.5% (w/w). Therefore, in order to get a reasonable response from the applied polymers and surfactants, samples were prepared in concentrations of 2% (w/v) by dissolving 40 mg of sample in 2.0 ml of mobile phase followed by sonication for 1 h to ensure complete dissolution.

### 2.5. Preparation of calibration standards

The preliminary experiments showed that the degree of surface adsorption differed from excipient to excipient. Therefore,

the concentration range of the calibration standards for the individual excipients was adapted to the excipient content in the microparticles.

Stock solutions of each of the excipients were prepared by dissolving the excipient in mobile phase containing 2% (w/v) of Lu 28–179. Dilutions were then carried out with mobile phase containing 2% (w/v) Lu 28–179 in such a way to ensure the concentration range covered the amount of excipient in each sample. It was not possible to dissolve HPMC by simply adding it to the mobile phase. Instead stock solutions of HPMC 4000 and 100,000 cP of 200 μg/ml were prepared by dissolving 20 mg of the polymers in 50 ml ammonium formate 20 mM pH 6.5 under stirring conditions at 80 °C. Once the solutions had been cooled to room temperature, acetonitrile was slowly added to a total volume of 100 ml. Lu 28–179 was added to the stock solution to a concentration of 2% (w/v), and dilutions were carried out with mobile phase containing 2% (w/v) Lu 28–179 as described above.

## 3. Results and discussion

### 3.1. Method development

#### 3.1.1. Separation

The analytical column was a mixed mode column, where the mechanism of separation is size exclusion, distribution/adsorption or ion exchange depending on the eluent conditions. In this case, the primary mechanism was size exclusion, which effectively separated all of the excipients from the drug substance. Fig. 2 shows chromatograms of HPC MW 95,000, HPMC 100,000 cP, Poloxamer 188, Brij 35 and SLS from samples of antisolvent precipitated particles. As can be seen from the chromatogram with SLS (2e), some partitioning also took place, as the hydrophobic drug molecules were retained for a longer period of time than SLS. The molecular weight of SLS is lower than that of the drug, and therefore, SLS would be expected to elute after the drug. The molecular weights and retention times of the excipients are listed in Table 1. The molecular weight cut off of the column was 40,000. Therefore, HEC, HPMC and HPC eluted as single sharp peaks in the void volume at approximately 8.2 min. However, this did not affect the quantification of the total amount of surface-adsorbed polymer.

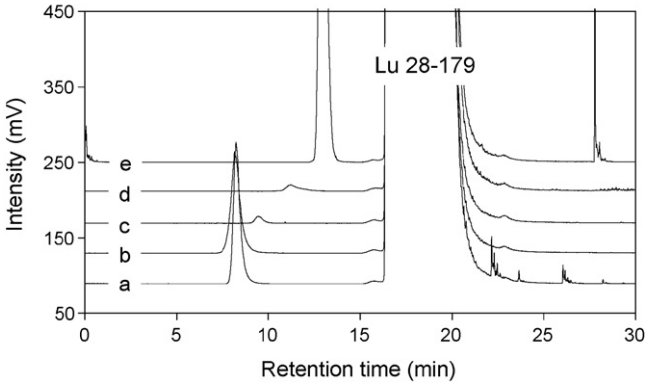


Fig. 2. Overlaid chromatograms from analysis of five excipients present at the surface of microparticles of Lu 28–179 prepared by precipitation: (a) HPC MW 95,000, (b) HPMC 100,000 cP, (c) Poloxamer 188, (d) Brij 35 and (e) SLS. Chromatograms are offset by 40 mV.

With Brij 35 (2d), MW 1200, some peak broadening was seen which might be attributed to chain length variations or secondary interactions with the packing material.

#### 3.1.2. Chromatographic conditions

The mobile phase composition of 50% organic and 50% aqueous solvent was chosen primarily based on the ability to dissolve both the drug and the excipients, and also based on the ability to elute the hydrophobic drug molecule from the column. As organic solvent, acetonitrile was chosen over methanol, as methanol was unable to elute the drug over a period of 2 h. A volatile electrolyte compatible with ELSD was added to the mobile phase to suppress ionic interactions between the analytes and the stationary phase. A mobile phase flow-rate of 0.6 ml/min was chosen, as it provided effective separation of the excipients from the drug and a low level of baseline noise.

### 3.2. Method qualification

#### 3.2.1. Specificity

The sample solutions contained Lu 28–179 and the excipient to be determined dissolved in mobile phase. To mimic this matrix, calibration standards for each excipient were prepared in mobile phase containing 2% (w/v) Lu 28–179. As illustrated in Fig. 3, no interferences were observed in the range of reten-

Table 1
Molecular weights, retention times and regression results from the evaluation of linearity

| Excipient | Molecular weight | Retention time (min) | Regression equation | $R^2$ | Concentration range (μg/ml) |
|---|---|---|---|---|---|
| Poloxamer 188 | 8,000 | 9.45 | $y = 1.63x + 3.67$ | 0.9987 | 5–25 |
| PEG | 6,000 | 9.82 | $y = 1.67x + 3.51$ | 0.9989 | 5–25 |
| Brij 35 | 1,200 | 11.19 | $y = 1.67x + 3.54$ | 0.9997 | 5–25 |
| HEC | 300,000 | 8.22 | $y = 2.06x + 2.66$ | 0.9985 | 5–25 |
| HPMC 4000 cP | [a] | 8.17 | $y = 1.25x + 3.81$ | 0.9968 | 25–200 |
| HPMC 100,000 cP | [a] | 8.17 | $y = 1.37x + 3.51$ | 0.9981 | 25–200 |
| HPC MW 95,000 | 95,000 | 8.16 | $y = 1.49x + 3.70$ | 0.9994 | 25–200 |
| HPC MW 850,000 | 850,000 | 8.25 | $y = 1.53x + 3.62$ | 0.9989 | 25–200 |
| SLS | 288 | 12.98 | $y = 0.80x + 5.42$ | 0.9915 | 210–350 |

The regression curves were obtained by plotting the logarithm of the peak area as a function of the logarithm of the analyte concentration.

[a] The molecular weights of the HPMCs were not given by the supplier. The viscosities of the polymers in 2% (w/w) aqueous solution were 4000 and 100,000 cP.

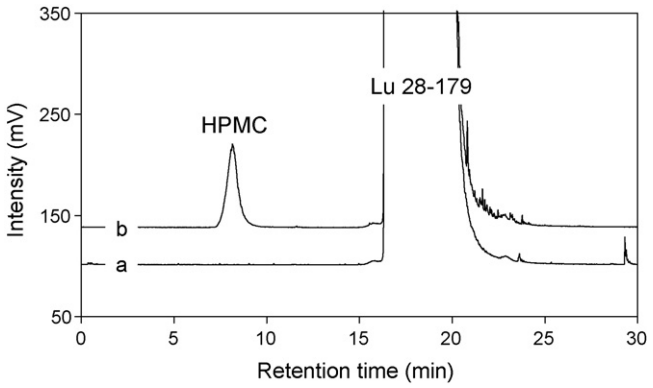


Fig. 3. Method specificity: (a) injection of a 2% (w/v) solution of Lu 28–179 in mobile phase and (b) injection of an HPMC 4000 cP standard solution (150 μg/ml) containing the polymer in a 2% (w/v) solution of Lu 28–179 in mobile phase.

tion times under study, when a blank containing 2% (w/v) Lu 28–179 in mobile phase was injected.

### 3.2.2. Linearity

It is well recognized that the ELSD gives a non-linear response as a function of the analyte concentration. A sigmoidal curve is observed with a linear section in the middle [7]. At the particle sizes predominating in most ELSDs, the main scattering mechanisms are reflection and refraction. The decrease in sensitivity at low concentrations of analyte is due to interference effects typical of Mie scattering occurring when the particle size is small, which causes the deflected light to be low in intensity at the measuring angles. When the analyte concentration is increased, a point is reached where the reduction in surface ratio of the particles to the particle concentration causes a proportionally smaller amount of light to be reflected and refracted, thus decreasing the sensitivity [7]. At the analyte concentrations normally applied, an exponential calibration curve is observed which can be described by Eq. (1):

$$Y = a \times m^b \quad (1)$$

where $Y$ is the response of the ELS detector, $m$ the mass or concentration of the analyte and $a$ and $b$ are constants determined principally by the nature of the mobile phase and the detector parameters, such as nebulizer design and wavelength of the light source [7,19,25]. If linearity exists between the detector response and the analyte mass or concentration, the constant $b$ is equal to 1.00. With the ELS detector $b$ is normally between 1 and 2 and can be determined by plotting the logarithm of the peak area against the logarithm of the analyte concentration ($\log Y = b \times \log m + \log a$) [18,19].

Such plots were used to evaluate the linearity of the responses. Calibration curves were constructed for each of the excipients containing five concentrations. For a given concentration either 2 or 6 replicates were performed according to the pattern 6-2-6-2-6. The concentration ranges for the individual excipients were based on preliminary determinations of the excipient contents in the nine different samples. Table 1 shows regression results for the excipients under study. As indicated by the regression

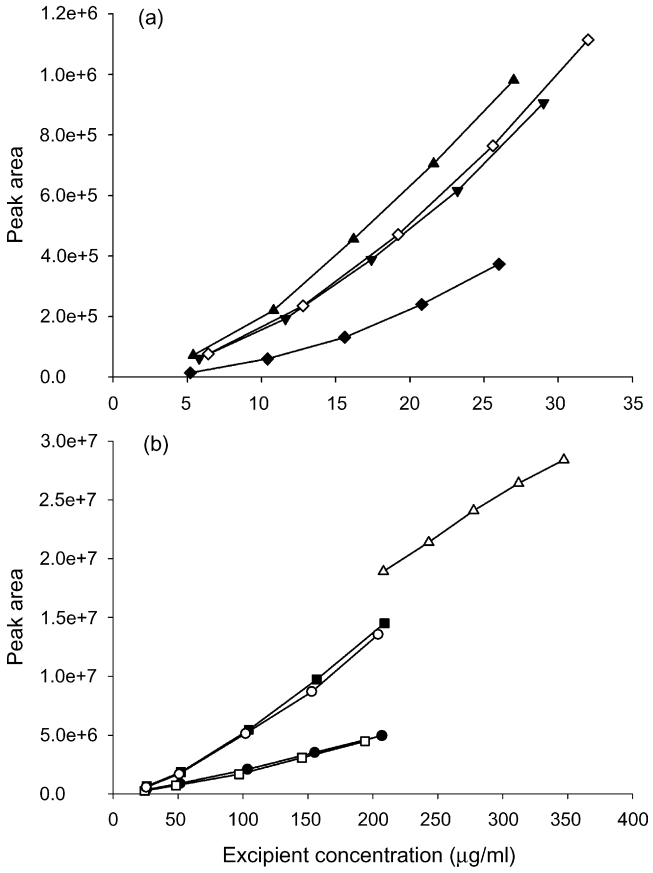


Fig. 4. Calibration curves for the excipients under study: (a) (▲) Poloxamer 188, (▼) PEG, (◊) Brij 35, (♦) HEC and (b) (●) HPMC 4000 cP, (□) HPMC 100,000 cP, (■) HPC MW 95,000, (○) HPC MW 850,000, (△) SLS.

coefficients, linearity was achieved by the log–log modelling. In contrast, none of the $b$-values are equal to 1, reflecting a non-linear direct relationship between analyte concentration and response (Fig. 4). The three different concentration ranges used for the calibration curves, allow a comparison of $b$-values obtained in the different concentration ranges. The results suggest that the $b$-values may be concentration dependent. Even though only one $b$-value was determined for each excipient, there is a tendency that the $b$-values determined for excipients calibrated in the intermediate concentration range of 25–200 μg/ml (HPMC 4000 and 100,000 cP and HPC MW 95,000 and MW 850,000) are closer to 1, than $b$-values determined for the excipients in the low range; 5–25 μg/ml for Poloxamer 188, PEG, Brij 35 and HEC. These values are closer to 2, whereas the $b$-value for SLS, calibrated in the range 210–350 μg/ml is below 1. An explanation for this could be that the intermediate concentration range 25–200 μg/ml contains a larger proportion of calibration point in the linear section of the sigmoidal curve and thus gives $b$-values for each individual compound closer to 1 compared to the 5–25 μg/ml range. This range represents the lower end of the sigmoidal curve, whereas the 210–350 μg/ml range may represent the upper end.

Fig. 4 also illustrates an important point regarding the dependence of the response on the structural characteristics of the analyte. If the response was dependent only on the analyte

concentration, the mobile phase composition and the detector parameters, all calibration curves in a given concentration range would be identical, provided that the analytes were injected in the same concentration and that the retention times were similar. This is not the case, however, and the reason can be attributed to differences in surface tension of the various excipients [23,24]. Most of the tested excipients are surface active, which cause them to influence the size of the formed droplets and thus the particles formed in the detector. The calibration curves for the two HPMCs are very similar, as are the curves for the two HPCs. However, due to structural differences, there is a great difference between the two HPMC-curves and the two HPC-curves, even though they elute at the same time.

### 3.2.3. Measurement precision

The measurement precision was evaluated for each excipient as three concentrations on each calibration curve were injected six times. Precision expressed as %R.S.D. was calculated for each of these six points and varied between 0.52 and 6.83 indicating good reproducibility of the method. The found precisions are comparable to measurement precisions previously determined for some of the excipients under study, e.g. 3.6% for HPMC, 1.3% for HPC and 1.1–1.5% for Poloxamer 188 [7,9,15].

### 3.2.4. Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were estimated as signal-to-noise ratios of 3 and 10, respectively (Table 2). ELSD is known to give LODs around 1 μg/ml [18], and all of the estimated LODs were also around 1 μg/ml. However, noticeable improvement was observed compared to previously reported methods for HPC, Poloxamer 188 and SLS. With the method developed for HPC by Zhu et al. [7], an absolute LOQ of 2 μg was determined compared to 0.45 μg for HPC MW 95,000 and 0.62 μg for HPC MW 850,000 with the method presented here. An absolute LOD of 5 μg for SLS has previously been achieved [12] compared to 0.07 μg in this study. A relative LOD of 8 μg/ml has been reported for Poloxamer 188 [15] compared to 1.1 μg/ml with the method presented here. The compound detected in the lowest concentration was SLS. Of the studied excipients, this compound eluted as the sharpest peak. This may be explained by the fact that SLS is a small compound of well-defined structure and molecular weight compared to the other excipients, which, although Brij 35 and Poloxamer 188 may also be classified as surfactants, are polymeric in structure, and therefore, may be subject to variation in chain length.

Table 2
Limits of detection and quantification defined as 3 and 10 times the signal-to-noise ratio

| Excipient | Relative | | Absolute | |
|---|---|---|---|---|
| | LOD (μg/ml) | LOQ (μg/ml) | LOD (μg) | LOQ (μg) |
| Poloxamer 188 | 1.1 | 3.7 | 0.11 | 0.37 |
| PEG | 1.7 | 5.6 | 0.17 | 0.56 |
| Brij 35 | 1.1 | 3.5 | 0.11 | 0.35 |
| HEC | 4.3 | 14.4 | 0.43 | 1.44 |
| HPMC 4000 cP | 3.0 | 10.0 | 0.30 | 1.00 |
| HPMC 100,000 cP | 3.2 | 10.5 | 0.32 | 1.05 |
| HPC MW 95,000 | 1.4 | 4.5 | 0.14 | 0.45 |
| HPC MW 850,000 | 1.9 | 6.2 | 0.19 | 0.62 |
| SLS | 0.7 | 2.4 | 0.07 | 0.24 |

Table 3
Degree of surface adsorption of excipients to drug particles

| Excipient | Excipient content of microparticles (μg/mg powder[a]) | Excipient percentage of microparticles (w/w) |
|---|---|---|
| Poloxamer 188 | 0.65 | 0.07 |
| PEG | None detectable | None detectable |
| Brij 35 | 0.92 | 0.09 |
| HEC | 1.13 | 0.11 |
| HPMC 4000 cP | 10.19 | 1.02 |
| HPMC 100,000 cP | 10.29 | 1.03 |
| HPC MW 95,000 | 5.15 | 0.52 |
| HPC MW 850,000 | 7.12 | 0.68 |
| SLS | 13.89 | 1.39 |

[a] Forty milligrams of powder was dissolved in 2.0 ml of mobile phase prior to analysis.

### 3.3. Determination of excipients adsorbed to the surface of drug particles prepared by antisolvent precipitation

Microparticles of the model drug Lu 28–179 were prepared by antisolvent precipitation in the presence of each of the excipients as described in Section 2. During precipitation the amount of excipient present equalled 10% (w/w) with respect to the drug. Following isolation, the amount of excipient adsorbed to the surface of the particles was determined; results are summarized in Table 3. The degree of excipient adsorption ranged from 1.39% (w/w) to as low as 0.07% (w/w) of the total particle weight, which is low compared to the amount of excipient present during precipitation. Thus, particles of very low excipient:drug ratios were obtained, which is desirable from a safety point of view. Even though the excipient weight percentage is low, each particle may be coated with a great number of excipient molecules, since the excipients are present only at the particle surface. In the case of the drug particles investigated in this study, the surface coatings provided by some of the excipients were sufficient to greatly alter the surface characteristics of the particles (unpublished data).

## 4. Conclusion

A rapid and simple method for the determination of various excipients in drug particles prepared by antisolvent precipitation was developed and qualified. Each excipient was baseline separated from the drug by size exclusion chromatography and quantified by ELSD. Limits of detection and quantification were below 4.3 and 14.5 μg/ml, respectively, and precision expressed as %R.S.D. was below 7%. The method was applied for determination of surface-adsorbed excipients to particles of the model drug Lu 28–179. Results showed that the degree of surface adsorption to the drug differed greatly during antisolvent precipitation, depending on the structure of the excipients. Others who wish to produce particles in a similar manner may also expect

this, and therefore, they may find it necessary to test a number of different excipients. The fact that the presented method is rapid and has low cost makes it very suitable for such screening purposes. Further, it can potentially be used for the simultaneous determination of Lu 28–179 and one or more of the tested excipients in other formulations, provided that they are present in comparable concentrations. Since the size of Lu 28–179 is typical of an organic drug substance, the method is likely to work for separation and determination of the tested excipients and other drug substances.

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