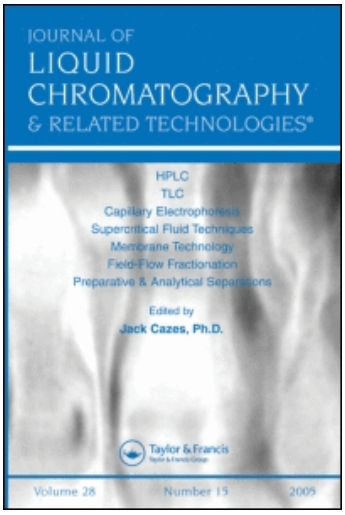

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Validated HPLC Method for the Determination of Residues of Acetaminophen, Caffeine, and Codeine Phosphate on Swabs Collected from Pharmaceutical Manufacturing Equipment in Support of Cleaning Validation†

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Abstract: A high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of residues of acetaminophen (paracetamol), caffeine, and codeine phosphate on swabs collected from pharmaceutical manufacturing equipment surfaces. Any residues of the compounds remaining on process equipment after cleaning are removed by swabbing with wet Texwipe® swabs, premoistened with methanol/water, followed by dry Texwipe® swabs. These residues are extracted from the swabs by means of an ultrasonic bath and the amounts of the compounds are determined. The chromatography was performed in the isocratic mode on a RP-18 column using a mobile phase consisting of 25 mM ortho-phosphoric acid and acetonitrile (90:10, v/v). UV- and fluorescence detection was performed in order to improve the method's sensitivity.

The method was validated by specificity, linearity, limit of detection, and limit of quantification, accuracy, and precision for the residues of acetaminophen (paracetamol), caffeine, and codeine phosphate on equipment surfaces. Stability studies have

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demonstrated the stability of the residual active compounds on equipment surfaces and on the swabs.

Keywords: HPLC, Method validation, Cleaning validation, Residues, Acetaminophen (paracetamol), Caffeine, Codeine phosphate

INTRODUCTION

Pharmaceutical manufacturing equipment has to be cleaned after production in order to avoid cross contamination in the next batch of a different product. The effectiveness of the cleaning process has to be confirmed by cleaning validation, which involves sampling and testing for acceptable residues on the pharmaceutical manufacturing equipment.[1–4]

There are two main types of sampling that have been found acceptable: The most desirable direct surface sampling of the equipment by using swabs and the use of the final rinse solution.[3] The testing for residues can involve specific methods, which are be preferred by the FDA, such as high performance liquid chromatography (HPLC), ion chromatography, atomic absorption, inductively coupled plasma, UV spectroscopy, ion selective electrodes, and enzymatic detection, or it can involve nonspecific methods such as total organic carbon, pH, and conductivity.

The design of a suitable sampling procedure and analytical method is very important in cleaning validation. The technique must be appropriate for measuring the analytes at and below the residue acceptable limit (RAL). Typical acceptance criteria used in the pharmaceutical industry are the 1/1000 dosage criteria (1/1000 reduction of the lowest therapeutic dose of the previous drug product), the 10 ppm criteria (no more than 10 ppm of any active pharmaceutical ingredient will appear in the next product), or the visual clean criteria (no residue will be visible on the equipment after the cleaning process).[5,6]

The main objective of this work is to develop a simple and selective HPLC method for the simultaneous determination of residues of acetaminophen (paracetamol), caffeine, and codeine phosphate on swabs collected from pharmaceutical manufacturing equipment surfaces after production of Azur® composition tablets and cleaning of the equipment.

Acetaminophen (paracetamol) and its combinations with other drugs like caffeine and codeine phosphate are very popular analgesic and antipyretic drugs and listed in the European and United States Pharmacopoeia[7,8] (for the chemical structures see Fig. 1).

Liquid chromatographic analysis with UV or fluorescence detection of analgesics has been carried out for decades and is widely used for production control of various dosage forms.[9,10] However, the challenge in cleaning validation is to develop analytical methods, which are sensitive enough to detect traces of the active compounds that remain on the surface of pharmaceutical

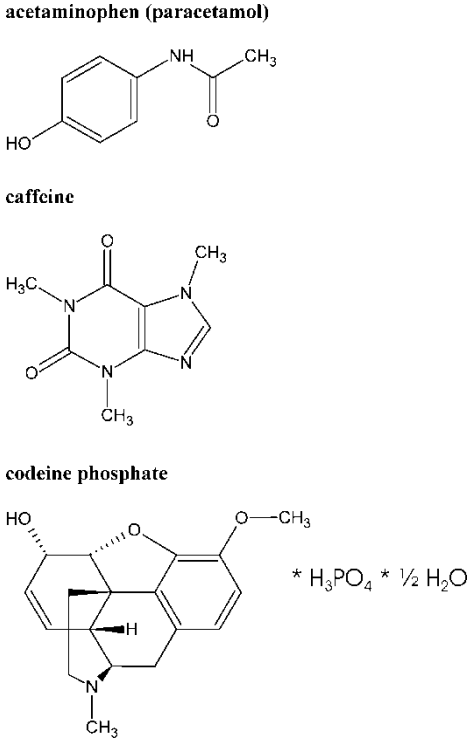

Figure 1. Chemical structures of the three active compounds.

manufacturing equipment after cleaning. In this case the RAL on the basis of the 1/1000 dosage criteria was calculated to 5.024 $\mu g/cm^2$ for acetaminophen, 0.754 $\mu g/cm^2$ for caffeine, and 0.151 $\mu g/cm^2$ for codeine phosphate. Therefore, an existing in-house HPLC method, which is used to quantify the amount of acetaminophen, caffeine, and codeine phosphate in Azur® composition tablets, was modified to improve the method's sensitivity for the cleaning validation assay.

The resulting HPLC method reported here has been validated in accordance to the ICH guidelines on the validation of analytical methods Q2A and Q2B.[11]

EXPERIMENTAL

Chemicals

Methanol and acetonitrile, both of HPLC gradient grade, and ortho-phosphoric acid (85%), Suprapur grade, were obtained from Merck (Darmstadt, Germany). Water used was purified by a Milli-Q academic water purification system (Millipore, Eschborn, Germany).

The active compounds, acetaminophen (paracetamol), caffeine, and codeine phosphate and the excipients microcristalline cellulose, copolyvidone, crospovidone, magnesium stearate, silicon dioxide are approved in-house substances (Steiner and Co., Berlin, Germany).

HPLC Equipment

The HPLC system (Waters, Eschborn, Germany) was an Alliance 2695XE separation module with solvent degasser, temperature controlled sample compartment and column heater, and a 2996 photodiode array detector coupled in line to a 470 fluorescence detector. The UV detection of the compounds of interest was carried out at 210 nm and the UV spectra were taken in the range of 210–400 nm. Fluorescence detection was performed at excitation 245 nm and emission 345 nm, respectively, and the gain was $\times 1000$.

For system control, data acquisition, and data processing, the Empower client/server software (Waters, Eschborn, Germany) was used. Statistical analysis was calculated using the MVA statistical software (Novia, Saarbrücken, Germany).

Chromatographic Conditions

LC separation was carried out using a Synergi Hydro RP-18 column with the dimension 150 × 3 mm, and 4 μm particle size (Phenomenex, Aschaffenburg, Germany). All chromatographic experiments were performed in the isocratic mode. The mobile phase was 25 mM ortho-phosphoric acid and acetonitrile (90:10, v/v). The flow rate was set to 0.5 mL/min and the column temperature to 30°C. The injection volume was 10 μL.

Standards Preparation

The reference standards of acetaminophen (paracetamol), caffeine, and codeine phosphate were approved in-house standards.

A stock solution containing 5.25 mg/mL acetaminophen, 0.75 mg/mL caffeine, and 0.45 mg/mL codeine phosphate was prepared in methanol/water (1:1, v/v). Dilutions were prepared in methanol/water (1:1, v/v) at ten concentration levels.

Collecting Samples

A plate of the same equipment construction material (stainless steel) was used, which has predefined 5 cm × 5 cm squares. Each square of the plate was spiked with known amounts of the analytes and allowed to dry.

For the swabbing, the very clean low background Texwipe-Swabs TX714A were used, which are available in kits with clean 40 mL sample vials (ITW Texwipe, Mahwah, USA).

A swab, moistened with methanol/water (1:1, v/v), was typically wiped over the defined square in a systematic way with side-by-side strokes vertically and horizontally. The procedure was repeated with a dry swab.

The heads of the two swabs were cut and transferred into the sample vial. Methanol/water (8 mL) (1:1, v/v) was added and the vial was placed in an ultrasonic bath for 10 min. An aliquot of the preparation was filtered through a 0.45 μm PTFE-membrane filtration cartridge (Gelman Sciences, Dreieich, Germany) directly into an auto sampler vial and transferred into the HPLC system.

Validation Study

In accordance with the ICH-Guidelines on the validation of analytical methods Q2A and Q2B the following validation characteristics were examined: specificity, linearity, limit of detection, and limit of quantification, precision, and accuracy.

RESULTS AND DISCUSSION

Method Development

An existing in-house HPLC method, which is used to quantify the amount of acetaminophen, caffeine, and codeine phosphate in Azur® composition tablets, was modified and optimised for a shorter run time by using a shorter column length. In addition, the amount of the organic solvent was increased.

The initial method detects the three compounds of interest at a wavelength of 210 nm. To improve the method's sensitivity for the cleaning validation assay, codeine phosphate was detected with fluorescence detection at an excitation wavelength of 245 nm and an emission wavelength of 345 nm.

A typical UV and FL chromatogram for the optimised method is given in Fig. 2.

Specificity

The ability of this method to separate the peaks of interest indicates the specificity of the method. Retention times and UV spectra of the reference substances were used to identify the peaks in the chromatograms. With the current separation conditions, acetaminophen elutes at 4 min, codeine phosphate at 5 min, and caffeine at 8 min.

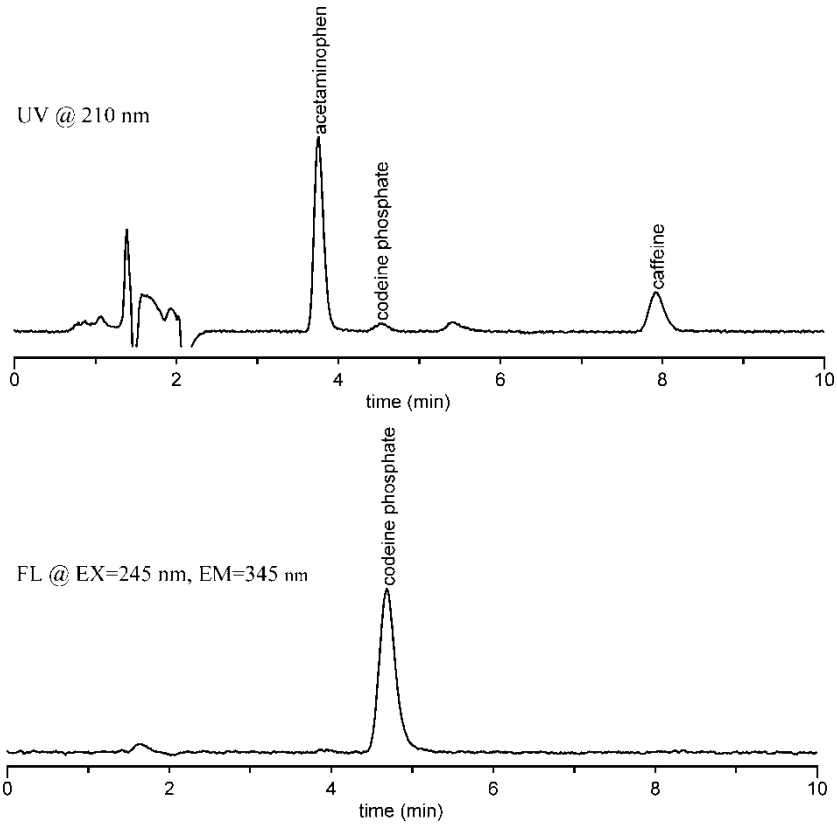

Figure 2. Representative chromatogram of the three residual compounds after the swab test. The concentrations are 2.6 μg/mL for acetaminophen, 0.4 μg/mL for caffeine, and 0.2 μg/mL for codeine phosphate. For chromatographic conditions, see text.

There is no interference from the extracted blank swabs, extraction solvent, and excipients used for the drug product Azur® composition tablets in the retention times of the three compounds of interest. Figure 3 shows chromatographic overlays of these injections.

Linearity

The linearity of the method was established by injection of standard solutions at ten concentration levels over a wide concentration range. The peak area versus concentration data was treated by linear regression analysis and the limits of detection (LOD) and quantification (LOQ) were calculated from the 95% estimation interval of the calibration line. The coefficients of correlation ($r > 0.999$) demonstrated the excellent relationship between

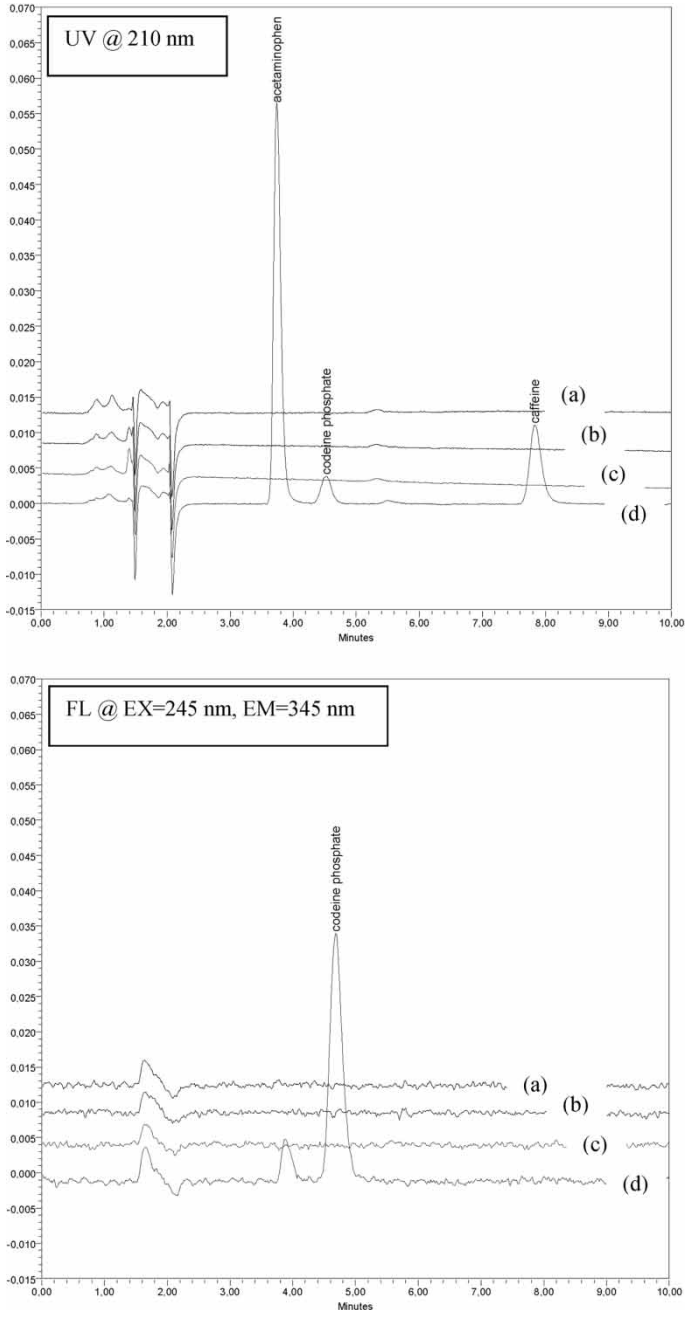

Figure 3. Overlay chromatograms of extracted blank swabs (a), extraction solvent (b), excipients used for the drug product (c), and the three active compounds (d). For details, see text.

peak area and the concentration of acetaminophen, caffeine, and codeine phosphate. For the calibration range, regression equation, the coefficient of correlation, and the LOD, LOQ for each of the three active ingredients see Table 1.

Precision of the Chromatographic Method

The precision of the HPLC method was performed at two different levels, repeatability and intermediate precision. The repeatability ("intra-assay precision") was determined by analysing six repeated injections of the standard solution (n = 6). For the intermediate precision, six determinations were repeated on a different day by a different analyst.

The relative standard deviation values of the repeatability and intermediate precision were less than 2% and illustrated that the HPLC method is precise (Table 2).

Precision of the Swab Test

The precision of the swab test was also performed at the two different levels, repeatability and intermediate precision. For determination of the repeatability ("intra-assay precision"), the solution of the active ingredients was spiked onto squares of the stainless steel plate by pipetting 100 μL of the solutions with the appropriate concentrations. After allowing the solution to air-dry, the swab test was performed. Six spiked squares were prepared and swabbed (n = 6).

For the intermediate precision, six determinations were repeated on a different day by a different analyst. The validation data in Table 2 show that the precision of the swab test is acceptable.

Table 1. Overview of the linearity data for the three compounds

	Analyte (API)		
	Acetaminophen	Caffeine	Codeine phosphate
Calibration range [μg/mL]	0.11–26.29	0.02–3.75	0.01–2.23
y-intercept	4185	1045	8612
Slope	58592	132830	2540762
Coefficient of correlation	0.99999	0.99993	0.99999
LOD [μg/mL]	0.156	0.056	0.011
LOQ [μg/mL]	0.233	0.083	0.016

Table 2. Precision and accuracy of the method

Validation parameter	Acetaminophen	Caffeine	Codeine phosphate
Precision of the HPLC method			
Repeatability (intra-day precision)	0.59%	0.60%	1.63%
Intermediate precision	0.52%	0.97%	1.64%
Precision of the swab technique			
Repeatability (intra-day precision)	5.22%	2.03%	5.06%
Intermediate precision	6.99%	2.90%	9.25%
Accuracy over recovery			
Average recovery	80.79%	90.21%	85.45%
95% Confidence interval	±2.37%	±4.07%	±7.51%
Coefficient of variation	4.62%	7.11%	13.82%

Accuracy

The recovery rate of spiked active compounds on the stainless steel plate was determined to evaluate the accuracy of the method. At four concentration levels, samples were prepared in triplicate (n = 12), and each sample was injected twice and analysed according to the method previously described.

Table 3. Results of the stability studies

Validation parameter	Acetaminophen	Caffeine	Codeine phosphate
Stability (24 h)			
Standard solution (% of initial recovery)	99.4%	99.4%	101.0%
Analyte on surface (% of initial recovery)	101.3%	100.6%	100.5%
Analyte on swab (% of initial recovery)	99.7%	101.0%	100.5%
Analyte in swab solution (% of initial recovery)	99.9%	100.0%	100.4%
Stability (48 h)			
Standard solution (% of initial recovery)	100.2%	99.3%	101.9%
Analyte on surface (% of initial recovery)	100.8%	101.2%	101.1%
Analyte on swab (% of initial recovery)	99.5%	100.3%	101.3%
Analyte in swab solution (% of initial recovery)	99.8%	100.7%	100.3%

The results of the recovery experiments show that the method is accurate for acetaminophen, caffeine, and codeine phosphate with recovery rates of 81, 90, and 85%, respectively. The results are summarized in Table 2.

Sample Stability

The stability of the active ingredients in solution, on swabs, on the stainless steel plate, and in the extraction solvent, was measured after 24 and 48 h stored at room temperature. The results are summarized in Table 3 and demonstrated that the active compounds were stable over two days.

CONCLUSION

In conclusion, a simple to use high performance liquid chromatographic (HPLC) method to quantify residues of the active pharmaceutical ingredients acetaminophen (paracetamol), caffeine, and codeine phosphate on swabs, in support of cleaning validation of pharmaceutical manufacturing equipment, was developed. Validation studies show that the HPLC method is selective, linear, precise, and accurate. The method has a detection limit for acetaminophen, caffeine, and codeine phosphate of 0.55, 0.17, and 0.02 μg/ml, respectively. Recovery studies demonstrated the accuracy of the method and more than 80% of the active compounds were recovered from the stainless steel plates.

Stability studies at different steps of the sampling procedure show that acetaminophen, caffeine, and codeine phosphate are, at least, stable over the investigated 48 h.

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