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Method development and validation for trifluoroacetic acid determination by capillary electrophoresis in combination with capacitively coupled contactless conductivity detection (CE-C^4D)

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ABSTRACT

A method was developed to determine traces of trifluoroacetic acid as impurity in synthetic or semi-synthetic drugs as antibiotics, macropeptides, etc. Capillary electrophoresis in combination with capacitively coupled contactless conductivity detection (CE-C^4D) was used due to lack of UV absorbance property of trifluoroacetic acid (TFA). The optimized method took less than 1 min with good linearity (R^2 = 0.9995) for trifluoroacetic acid concentration from 2 to 100 ppm. It also has a good repeatability expressed by the relative standard deviation (% RSD) which is 1.2 and 2.1% for intraday and interday precision, respectively, at 50 ppm TFA, and good sensitivity with 0.34 ppm, 1.2 ppm LOD and LOQ, respectively. In addition, the content of TFA in synthetic drug, was determined using the validated method which gave good linearity (R^2 = 0.9996) for trifluoroacetic acid spiked into drug in a concentration range of 2–80 ppm, with good intraday repeatability of 2.0%.

The analysis is performed in a background electrolyte composed of 20 mM morpholinoethane-sulfonic acid (Mes) and 20 mM L-histidine (L-His) pH 6.1. Cetyltrimethylammonium bromide (CTAB) was added as flow modifier in a concentration (0.2 mM) lower than the critical micellar concentration. Ammonium formate 6 ppm was used as internal standard. The applied voltage was 30 kV in reverse polarity. A fused silica capillary with 75 μm internal diameter and total length 47 cm (31 cm to C^4D detector and 37 cm to DAD detector) was used.

1. Introduction

Trifluoroacetic acid (TFA) has strong acidity (pKa = 0.3) due to the presence of three fluorine atoms. TFA is widely used in organic chemistry (synthesis). In the environment, trifluoroacetic acid results from the breakdown of different hydrochloro-fluorocarbons (HCFC's) and hydrofluorocarbons (HFC's) such as HCFC-123, HCFC-124 and HCFC-134a. Those byproducts contribute to the depletion of the ozone layer. Therefore environmental measures are taken such as the withdrawal of those compounds from being used as cooling agent [1].

During this research the field of investigation was the determination of trifluoroacetic acid as production impurity in a drug by using C^4D.

Trifluoroacetic acid is used as deprotection agent during solid phase peptide synthesis (SPPS) [2,3]. Peptides have nowadays a wide range of applications, and synthetic peptides are currently in great demand within pharmaceutical industry. Hormones such as oxytocin and calcitonin have been known for a long time and their roles in performing specific biochemical functions are well understood. More recently discovered peptides include enkephalins such as Met-enkephalin and Leu-enkephalin. These are neuroactive peptides that belong to a new class called opioid peptides. Peptides such as calcitonin and secretin are medicinally important as therapeutic agents and are manufactured on a large scale [4].

The development of new peptides as potential therapeutic agent is also growing rapidly. Peptides are also increasingly being used as model compounds in protein research. The need for synthetic peptides and also technological advances in peptide synthesis has grown dramatically over the past few decades with the rapid growth of newly discovered peptides and proteins.

Antibiotics are produced either naturally through fermentation (erythromycin, penicillin, etc.) by microorganism species or through semi-synthesis in the laboratory. The synthesis of particular antibiotics at the laboratory scale uses peptides which can contain traces of TFA, strong acid used during the deprotection process in the acidolysis of t-butyloxycarbonyl (BOC) groups or during the cleavage process of the covalent bond between the solid phase and the peptide [3].

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TFA is a toxic and corrosive substance. Due to that it can alter the biological actions or effects during clinical studies of synthetic peptides. Therefore, any residual TFA must be removed from peptides prior to the final formulation. Its removal must be reliably checked and measured in products intended for preclinical or clinical applications. So the development of reliable and efficient methods for determining acids such as trifluoroacetic acid in synthetic peptide samples is important. However, trifluoroacetic does not absorb in UV. Different modes of detection have been used for the determination of TFA such as ion chromatography (IC) using ion-exchange columns and conductivity detectors [5–12].

Application of IC was shown to be a straightforward method to determine TFA in peptide samples [9].

Nowadays capillary electrophoresis (CE) has become an efficient and reliable technique to determine organic anions. Comparing the two techniques IC and CE [13,14], CE has many advantages over IC for ion analysis in terms of selectivity, peak capacity, ease of adaptability and rapid analysis times.

But in CE, because the online detection window of the capillary is very small, indirect detection becomes an excellent choice for detecting analytes that have insufficient UV absorption to permit direct detection, such as formic acid and acetic acid. The feasibility of indirect detection of small organic acids as formic acid and acetic acid by CE was first demonstrated by Hjertén et al. [15] in 1987. Since then, analysis of acetic acid was successfully achieved by making use of the excellent separation capabilities of CE coupled to indirect detection [16–22]. In most of these cases, UV-absorbing background electrolytes such as chromate or phthalate and electroosmotic flow modifiers such as cetyltrimethylammonium bromide (CTAB) have been used to achieve good separation and detection of anions in various sample matrices [23].

Recently a steadily increasing number of reports have appeared, dealing with the application of capacitively coupled contactless conductivity detection (C^4D) [24–26]. This detection mode has been used for the analysis of inorganic, organic ions as well as biomolecules.

In this paper we describe a combination of capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) for the determination of trifluoroacetic acid in a drug.

In contrast to the different methods used so far for trifluoroacetic determination, this method uses a direct determination by measuring the conductivity change of the background electrolyte due to analyte presence. The conductivity change is related to the type and the analyte concentration in the flowing background electrolyte.

During this study we intend to validate the method through linearity, repeatability, inter and intra-day repeatability, limit of detection and limit of quantification.

2. Materials and methods

2.1. Reagents and sample

All chemicals used were of analytical grade. 2 (N-Morpholino)ethanesulfonic acid monohydrate (Mes), L-histidine (L-His) and ammonium formate were purchased from Fluka (Munich, Germany), TFA, formic acid and propionic acid came from Acros Organics (Geel, Belgium), N-cetyltrimethyl-ammonium bromide from Merck (Darmstadt, Germany), sodium hydroxide from Riedel-deHaën (Seelze, Germany), sodium chloride from Fischer chemicals (Leicestershire, UK) and sodium acetate from Applichem GmbH (Darmstadt, Germany). The drug sample originated from pharmaceutical industry.

All solutions used during the experiments were made with ultrapure MilliQ-water (Millipore Milford, MA, USA) and filtered through a 0.2 μm membrane filter (Dassel, Germany).

The pH value of buffers was measured and adjusted with the aid of a pH-meter Metrohm 691 (Herisau, Switzerland).

A stock solution of 1000 ppm TFA was prepared by dissolving an exact amount of trifluoroacetic acid in water. Solutions of different concentrations were obtained from this stock solution by dilution.

New capillaries were conditioned at 45 °C by rinsing with 1 M NaOH (10 min), 0.1 M NaOH (30 min), waiting for 30 min and water (5 min). Daily at the start of analysis, the capillary was rinsed with 1 M NaOH (5 min), 0.1 M NaOH (3 min), water (1 min) and BGE (2 min). During method development and method application to drug analysis, the capillary was rinsed with 0.1 M NaOH (1 min), water (1 min) and BGE (2 min). All steps being performed at 25 °C and 20 psi.

2.2. Instrumentation

In the present work, dual detection was used; UV detection for the UV absorbing drug and C^4D for TFA. The experiments were performed on a P/ACE MDQ instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a photo diode array detector coupled to a homemade C^4D system, with a TG315 function generator that delivers a frequency range of 100–3000 kHz. The sinusoidal wave from the internal oscillator is multiplied from 2 V_{P-P} to 20 V_{P-P} by an amplifier and it is applied directly to the excitation electrode of a homemade C^4D cell. The data were acquired by labView 8.5. Software (National Instruments), and further data treatment was done by 32 Karat™ 4.0 software. Uncoated fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). On-line UV detection was performed at 229 nm, the wavelength of maximum absorption of the tested drug.

2.2.1. Optimization of detector conditions

C^4D detection was carried out with a TG315 function generator, a high voltage contactless conductometric detector. The detector should be operated at its optimum frequency, which is as low as possible to minimize the effect of stray capacitance [26]. The highest signal for TFA and more stable baseline were found at 550 kHz, and 3.5 V_{P-P}.

3. Results and discussion

3.1. Method development

The aim of the study was the development, optimization and validation of a method for TFA determination in a drug. In order to achieve that goal, a relevant method development set up was needed.

The buffer pH as well as the ionic strength influence the electrophoretic mobility of the solutes. Hence the choice of the background electrolyte (BGE) constituents is crucial. The mixture of Mes and L-His also called “Good buffer” was used in combination with C^4D detection for keeping the background conductivity as low as possible. Mes and L-His both produce the necessary pH buffering, and a pH of 6.1 can be obtained by adding an appropriate volume of histidine solution to Mes solution while measuring the pH. CTAB was added to the mixture as flow modifier in a concentration less than the critical micellar concentration (CMC). The concentration of Mes, L-His and CTAB was varied during method development. Stock solutions of 100 mM of Mes, L-His and 2 mM of CTAB were prepared. Different combinations were prepared from the stock solutions and tested in order to define the best combination for the determination of trifluoroacetic acid presence in the drug. In fact, all the possible combinations of these three parameters at three levels (Table 1) were tested, resulting in 27 runs. The experiments were performed randomly so that systematic error could be avoided.

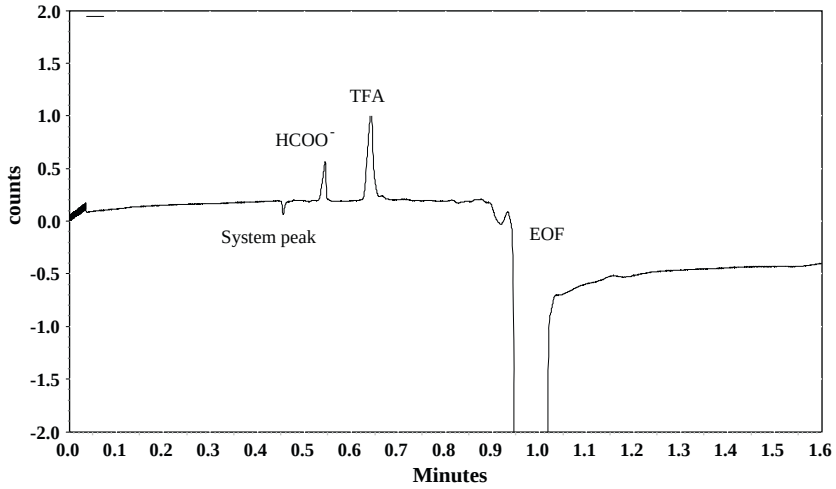

Fig. 1. Electropherogram of a reference solution of 50 ppm TFA with 6 ppm formate as I.S. using the developed method for TFA determination. Running background electrolyte: 20 mM Mes/L-His, 0.2 mM CTAB, pH 6.1, separation voltage −30 kV; hydrodynamic injection at 0.5 psi 0.5 s; C^4D detection.

The following responses were visually evaluated: the signal-to-noise ratio (S/N) of the TFA peak and its peak symmetry. The combination 20 mM Mes/L-His and 0.2 mM CTAB, pH 6.1 was retained because it gave the highest signal-to-noise ratio as well as a symmetric peak.

The internal standard (IS) was chosen among different compounds (sodium chloride, propionic acid, sodium acetate and ammonium formate) which were tested during method development. Propionic acid was found to co-migrate with trifluoroacetic acid and the chloride ion co-migrated with the system peak (see Fig. 1) as they have almost the same mobility. The acetate anion was well separated from TFA. Even better results were obtained with ammonium formate, which gave the best resolution. Therefore, it was retained as internal standard. Fig. 1 shows a typical electropherogram when operated under the adopted conditions, with TFA well separated in less than 1 min.

3.2. Method validation

As discussed above, only one of the tested anions co-migrated with TFA and the method was selective for the other anions.

Several quantitative aspects such as detection and quantification limits, linearity and repeatability were examined according to the guidelines issued by the International Conference on Harmonisation (ICH) for the validation of analytical procedures [27].

3.2.1. Linearity and sensitivity for pure TFA

The linearity was checked in triplicate, with ammonium formate as internal standard (6 ppm) at 6 concentration points for TFA (2, 5, 10, 20, 50 and 100 ppm). For calculation, corrected peak areas were used. The following regression equation was obtained: $y = 0.0715x - 0.0944$, with $R^2 = 0.9995$.

LOD and LOQ values corresponding to 3 and 10 times the S/N were 0.34 ppm and 1.2 ppm, respectively.

Table 1
Factorial analysis.

Electrophoretic parameters	Low value (−)	Central value (0)	High value (+)
Mes (mM)	20	30	40
L-His (mM)	20	30	40
CTAB (mM)	0.2	0.3	0.4

The evaluated responses were: (1) the signal-to-noise ratio (S/N); (2) the symmetry of the peak.

3.2.2. Repeatability for pure TFA

The repeatability of the optimized method was expressed as the relative standard deviation (% RSD) of the relative area. The intraday precision was found to be 1.3, 1.2 and 0.9% for 2, 50 and 100 ppm TFA solutions, respectively ($n = 6$, in one day, $n = 16$ for 50 ppm) and the interday precision of 50 ppm was 2.1% ($n = 48$, 16×3 days).

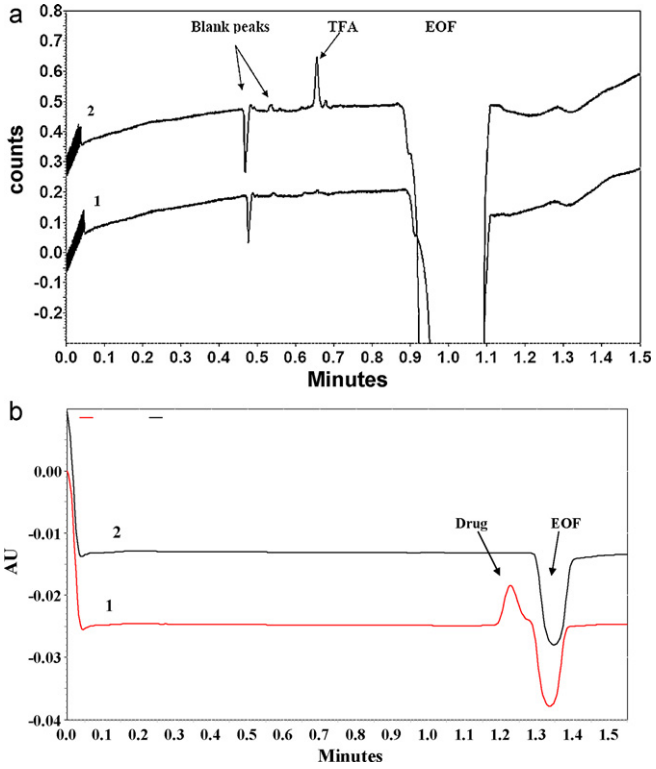

Fig. 2. (a) Electropherogram for TFA determination in a drug; background electrolyte: 20 mM Mes/L-His, 0.2 mM CTAB, pH 6.1, reverse polarity, −30 kV; hydrodynamic injection at 0.5 psi 0.5 s; C^4D detection. (1) Drug of interest at a concentration of 2.1 mg ml^{-1} No TFA was detected. (2) Drug spiked with 10 ppm TFA. (b) Electropherogram for TFA determination in a drug; background electrolyte: 20 mM Mes/L-His, 0.2 mM CTAB, pH 6.1, reverse polarity, −30 kV; hydrodynamic injection at 0.5 psi 0.5 s, UV detection at 229 nm. (1) Drug of interest at a concentration of 2.1 mg ml^{-1}. (2) TFA at a concentration of 10 ppm.

3.2.3. Linearity, sensitivity and repeatability for TFA spiked into test drug

The determination of the linearity of TFA spiked into a test drug was performed in triplicate as mentioned above, at 6 concentration points for TFA (2, 5, 10, 20, 50 and 80 ppm) and a drug concentration of 2.1 mg ml^{-1}. The following regression equation was obtained: $y = 0.0633x - 0.0982$, with $R^2 = 0.9996$. The intraday repeatability of 50 ppm spiked TFA solution was 2.0%. The LOD and LOQ of TFA spiked into tested drug were 0.6 ppm and 2.0 ppm, respectively.

The percentage accuracy at four different concentrations (5, 10, 20 and 50 ppm) of TFA spiked into test drug was 88.4, 86.5, 88.7 and 89.1%, respectively.

4. Application of the optimized CE- C^{4}D method to the determination of TFA in a drug

The method was applied to the determination of trifluoroacetic acid in a drug sample. A solution of 2.1 mg ml^{-1} was investigated for the presence of trifluoroacetic acid traces. No trace of TFA was observed at this concentration in the sample (Fig. 2a). The drug itself migrates just before the EOF, as can be deduced from the DAD signal (Fig. 2b).

We also investigated the concentration effect by using concentrated drug solutions. Different solutions of increasing concentration of the drug were prepared (4.2, 6.3, 8.4 and 10.5 mg ml^{-1}) and centrifuged for 3 min at 10,000 rpm. The supernatant was directly injected. Electropherograms showed no traces of TFA in spite of the high amount of the drug used.

5. Conclusion

The goal of this work was the development, optimization and validation of a method for the determination of trifluoroacetic acid in a drug sample. The method described is fast because of a relatively short electrophoretic migration time (less than 1 min) for the internal standard and the trifluoroacetic acid.

This C^{4}D mode of detection can be useful for the analysis of substances such as small inorganic ions (halogens, alkali metals), alcohols, carbohydrates, proteins, etc. It can also be a good alternative to derivatization in case of non-UV-absorbing substances.

One of the features of this method is that it offers the possibility to determine in a single run both trifluoroacetic acid using capacitively coupled contactless conductivity detection and UV-absorbing substances in a drug (main compounds and related substances), being time and reagent saving. This advantage can be useful for the analysis of more complex substances.

The proposed method using dual detection can constitute a promising alternative to other methods with acceptable linearity, repeatability, sensitivity and specificity.

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References

[1] T.J. Wallington, W.F. Schneider, D.R. Worsnop, O.J. Nielsen, J. Sehested, W. DeBruyn, J.A. Shorter, Environ. Sci. Technol. 28 (1994) 320A–326A.
[2] Y. Shin, K.A. Winans, B.J. Backes, S.B.H. Kent, J.A. Ellmen, C.R. Bertozzi, J. Am. Chem. Soc. 121 (1999) 11684–11689.
[3] P.E. Dawson, M.J. Churchill, M.R. Ghadiri, S.B.H. Kent, J. Am. Chem. Soc. 119 (1997) 4325.
[4] K. Burgers, J. Ibarzo, S.D. Linthicum, D.H. Russell, H. Shin, A. Shitangkoon, R. Totani, A.J. Zhang, J. Am. Chem. Soc. 119 (1997) 1556–1564.
[5] H. Small, T.S. Stevens, W.C. Bauman, Anal. Chem. 47 (1975) 1801–1809.
[6] W.R. Jones, P. Jandik, A.L. Heckenberg, Anal. Chem. 60 (1988) 1977–1979.
[7] W.R. Jones, P. Jandik, M.T. Swartz, J. Chromatogr. 473 (1989) 171–188.
[8] N. Simonzadeh, J. Chromatogr. 634 (1993) 125–128.
[9] A.A. Ammann, T.B. Rüttimann, J. Chromatogr. A 706 (1995) 259–269.
[10] K. Fischer, H.-P. Bipp, D. Bieniek, A. Kettrup, J. Chromatogr. A 706 (1995) 361–373.
[11] G. Saccani, S. Gherardi, A. Trifirò, C.S. Bordini, M. Calza, C. Freddi, J. Chromatogr. A 706 (1995) 395–403.
[12] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445–458.
[13] W.R. Jones, P. Jandik, J. Chromatogr. 608 (1992) 385–393.
[14] K. Hettiarachchi, S. Ridge, J. Chromatogr. A 817 (1998) 153–161.
[15] S. Hjertén, K. Elenbring, F. Kilár, J.-L. Liao, A.J.C. Chen, C.J. Siebert, M.-D. Zhu, J. Chromatogr. 403 (1987) 47–61.
[16] J. Romano, P. Jandik, W.R. Jones, P.E. Jackson, J. Chromatogr. 546 (1991) 411–421.
[17] B.F. Kenney, J. Chromatogr. 546 (1991) 423–430.
[18] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. 549 (1991) 345–355.
[19] V. Levi, T. Wehr, K. Talmadge, M. Zhu, Am. Lab. (1993) 29–32.
[20] J.B. Nair, C.G. Izzo, J. Chromatogr. 640 (1993) 445–461.
[21] P.R. Haddad, A.H. Harakuwe, W. Buchberger, J. Chromatogr. A 706 (1995) 571–578.
[22] C.H. Wu, Y.S. Lo, Y.-H. Lee, T.-I. Lin, J. Chromatogr. A 716 (1995) 291–301.
[23] Y.G. Xiao, Applications of Capillary Electrophoresis with contactless conductivity detection, PhD thesis, Basel, 2008.
[24] A.Z. Carvalho, Use of capillary electrophoresis in combination with mass spectrometry, spectrophotometry or capacitively coupled contactless conductivity detection in the analysis of drug substances, PhD thesis, Leuven, 2009.
[25] A.Z. Carvalho, J.A.F. da Silva, C.L. do Lago, Electrophoresis 24 (2003) 2138–2143.
[26] P. Kuban, P.C. Hauser, Electrophoresis 30 (2009) 176–188.
[27] Q2 (R1) Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Humans Use: Geneva, 1994.