ELSEVIER

Journal of Chromatography A 817 (1998) 239–252

JOURNAL OF CHROMATOGRAPHY A

Characterisation of buserelin acetate by capillary electrophoresis

Hermann Wätzig*, Matthias Degenhardt

University of Würzburg, Institute of Pharmacy and Food Chemistry, Am Hubland, D-97074 Würzburg, Germany

Abstract

In order to characterise the side compounds of the anticancer peptide drug buserelin acetate down to 0.1 area%, the sensitivity-increasing field-amplified sample injection (FASI) of a sample with low conductivity has been used. The signal-to-noise ratio has been improved by the factor of 50. FASI is best used with long injection times (e.g. 20 s) and relatively low voltage (e.g. 1 kV). After all compounds of interest were detected, selectivity was optimised. Separation was investigated in the pH range from 2 to 12; all buffers with pH below 3.5 were suitable. The method was validated with respect to accuracy, precision, linearity, sensitivity, selectivity, sample solution stability and sample depletion. This purity test has also been found useful to study the behaviour of buserelin acetate implants under stress conditions. The increase of side components after sterilisation using γ-radiation can be determined. Three additional compounds are found after treatment by radiation, one in a content of less than 0.1 area%. These compounds are unstable. The developing products of decay are also found in samples that were not treated with γ-rays but stored for several months in a stability test. This has been shown by a new within-capillary experiment. The substances were separated, recollected by switching the pole and separated again after several hours. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Field-amplified sample injection; Injection methods; Buserelin acetate; Peptides

1. Introduction

Buserelin is an important drug which reduces the testosterone plasma level in order to treat prostate cancer. This nonapeptide (5-Oxo–Pro–His–Trp–Ser–Tyr–*tert.*-butyl-D-Ser–Leu–Arg–Pro–NH–C_2H_5; M_r=1299.5; isoelectric point, pI=10.5) is a synthetic analogue to the hypothalamus hormone gonadoliberin. Buserelin is by far more effective than the physiologically active agent. Thus the synthesis of testosterone is even increased during the first days of treatment. However, soon feedback regulation starts to suppress this additional testosterone production. The primary effect of buserelin is overcompensated, thus a long-term decrease of the testosterone plasma concentration is achieved [1].

Peptides can be made bioavailable by subcutaneous injections, by nasal application or by using implants. The latter is the method of choice to assure constant blood levels. However, implants must be sterile. Heat cannot be used to sterilise buserelin implants, because these consist of a special biodegradable polymer. Therefore γ-radiation should be the best method to sterilise these implants.

However, some questions must be answered before this method can be established. Is the drug within its formulation stable when exposed to γ-radiation? Are additional side compounds formed? Can radiation-exposed implants be distinguished from nonsterilised? This would be useful to prove that sterilisation of a product has actually taken place.

Capillary electrophoresis (CE) has been chosen to answer the above questions. This technique has

*Corresponding author.

0021-9673/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(98)00443-9

already been used to successfully characterise γ-radiation induced radical products of antipyrine [20].

The performance of CE as a powerful tool in peptide analysis has been demonstrated in a number of recent reviews, e.g., Refs. [2–4]. Peptide adsorption at the capillary wall can be effectively prevented using isoelectric buffers [2]. Fraction collection, possibilities to enhance the sensitivity and CE–MS coupling has been discussed [4]. Theoretical consideration can be found about the behavior of peptides in CE, depending on their charge, size and shape. The peptide mobility depends on the pH and the ionic strength of the buffer. Moreover, selectivity can be improved using surfactants and other additives [3,4].

2. Experimental

2.1. Instrumentation and analytical separation

The CE experiments were performed with P/ACE 2050 and 2100 systems (Beckman, Palo Alto, CA, USA). Fused-silica capillaries of 20 cm or 30 cm length (inlet to detector) and 25 μm I.D. have been obtained from Polymicro (Phoenix, AZ, USA). Prior to their first use they were conditioned with 0.1 *M* NaOH for 30 min, heating to 50°C, and then equilibrated with running buffer for 40 min under the subsequent running conditions. Before each run the capillary was rinsed with 0.01 *M* NaOH for 1 min and with the running buffer for 2 min. The thermostat was set to 30°C. The wavelength of detection was 214 nm. The separation voltage was 8 kV (≈100 μA, cathode at the outlet buffer) for the final method. In earlier experiments 10 and 15 kV had been used. The samples were electrokinetically injected (1 kV, typically 20 s).

Best separations were obtained using a phosphate buffer pH 3.0, 60 mmol/l containing 250 mmol/l K_2SO_4, prepared by making up 774 mg orthophosphoric acid (85% w/w), 7.252 g KH_2PO_4 and 43.56 g K_2SO_4 in 1000.0 ml of HPLC-grade water (Millipore, Eschborn, Germany). All chemicals were of analytical grade (Merck, Darmstadt, Germany).

The other tested buffers and electrolytes were phosphate (pH: 2.0, 2.5, 3.5, 5.5, 6.0, 6.5, 7.5), acetate (pH: 4.0, 4.5, 5.0), citrate (pH: 2.4, 3.0, 3.5, 4.0), glycinate (pH: 9.5, 10.0, 10.5, 11.0) and NaOH (pH 12.3). Recipes for these buffers can be found in Refs. [5,6]. The buffer concentrations were 60 mmol/l (except NaOH which was 20 mmol/l) additionally containing 250 mmol/l K_2SO_4.

2.2. Sample pretreatment

Two rod-shaped implants (about 10 min long, containing 3.3 mg buserelin acetate each) were solved in eight drops of dimethyl(formamide) (DMF) within a centrifuge vial using ultrasonication. After adding eight drops of water, polymer from the implant precipitates. After centrifuging (2 min, 10 000 rpm) the supernatant is taken with a pipette and cleaned by repeated centrifuging.

2.3. Investigation of subsequent decay reactions (compare Section 3.5)

2.3.1. Separation of side compounds from buserelin (compare Fig. 8A–C, below)

A CE separation is carried out as usual (cathode at outlet). This separation is stopped before the side compounds migrate out of the capillary. The stop time t_{stop} is calculated as

$$t_{stop} = t_{begin\ 1st} \cdot \frac{L}{l}$$

Here $t_{begin\ 1st}$ denotes the time corresponding to the begining of the first side compound peak, L and l are total and effective capillary length, respectively.

2.3.2. Back migration of side compounds (Fig. 8D, E)

The separation voltage is applied in the opposite direction (now: anode at outlet). The compounds pass the detector in reversed order; thereby the complete separation from buserelin is validated. The back migration is stopped after all side compounds have passed the detector and 90% of their migration time in Section 2.3.1 has gone by.

2.3.3. Study of products of decay

The capillary is allowed to stand for several hours. After reswitching the pole (outlet=cathode), side

compounds and their products of decay are separated (Fig. 8F, G).

3. Results and discussion

3.1. General considerations

In order to achieve sufficient selectivity, the standard method is to vary the buffer pH. If an analyte contains a number of acidic or basic groups, it is best to use standard screening buffers over a wide range and refine the pH in regions where separation is shown (e.g. Ref. [5]).

However, in the case of buserelin additional initial considerations become necessary. Buserelin is a basic peptide. Thus precautions are needed to avoid adsorption. Therefore a rinsing step with 0.01 *M* NaOH solution has been introduced to remove residual peptide molecules after each run. In order to decrease adsorption during analysis, a buffer of high ionic strength has been selected; potassium sulfate as major component has been used because of its very low UV absorbance [7].

A buffer of high ionic strength always means high conductivity. Therefore 25 μm capillaries have been used to avoid excessive Joule heating. The loss in sensitivity because of the shorter light path compared to 50 μm capillaries is usually compensated by higher peak efficiency obtained using narrower capillaries. Recently the use of isoelectric buffers has been suggested to avoid Joule heating [2].

Preliminary experiments showed good peak shape, sufficient separation efficiency (five peaks/min) and short analysis times (Fig. 1). However, sensitivity was insufficient, although the signal-to-noise ratio has been measured as approximately 300. No side compounds have been observed. However, in order to optimise a separation method, all analytes of interest must be detectable. Therefore, the method has first been improved with respect to sensitivity. Only after that an optimisation with respect to selectivity became possible.

Two possibilities have been used to improve sensitivity. Firstly, field-amplified sample injection (FASI) has been used. Secondly, the sample preparation was optimised such that only a minimum amount of solvent became necessary, leading to an increase in the sample concentration.

3.2. Improving sensitivity

3.2.1. FASI

FASI is an extension of sample stacking, which is often applied to improve the signal-to-noise ratio in CE. FASI means electrokinetic injection from sample solutions of low conductivity into capillaries filled with highly conducting buffers. When voltage is applied, the field strength is high in the sample solution between electrode and capillary inlet and lower within the buffer-filled capillary. Stacking takes place at the interface between sample solution and buffer. When sample stacking is applied after hydrodynamic injection, typically only a few nanoliter of sample solution are injected. Using FASI, in principle the whole content of a sample vial (e.g. 1 ml) can be accumulated at the interface. Thus the amplification effect is much higher than using simple stacking.

This technique was first demonstrated by Chien and Burgi [8,9]. Meanwhile its performance has been successfully confirmed and improved [10–16].

This analytical task was favourable for the use of FASI. A buffer of high conductivity was used, and the sample solution was an especially poor conductor due to the content of organic solvent. The result of the use of FASI is shown in Fig. 2. The signal-to-noise ratio was improved by the factor of 50 compared to the initial experiments.

In principle the injected sample amount can be increased by increasing the injection time and the injection voltage using electrokinetic injection. However, using FASI, it is favourable to prefer long injection times (e.g. 5 min) and use moderate voltages (e.g. 1 kV) [12]. The electric field in the sample vial is inhomogeneous (Fig. 2). The sample molecules reach the zone of high field strength between electrode and capillary not only by electrostatic attraction but also diffusion-controlled. The injected amount linearly increases with injection time. However, from a certain injection voltage on, the injected amount increases less than linearly with voltage due to sample depletion of the region of high field strength.

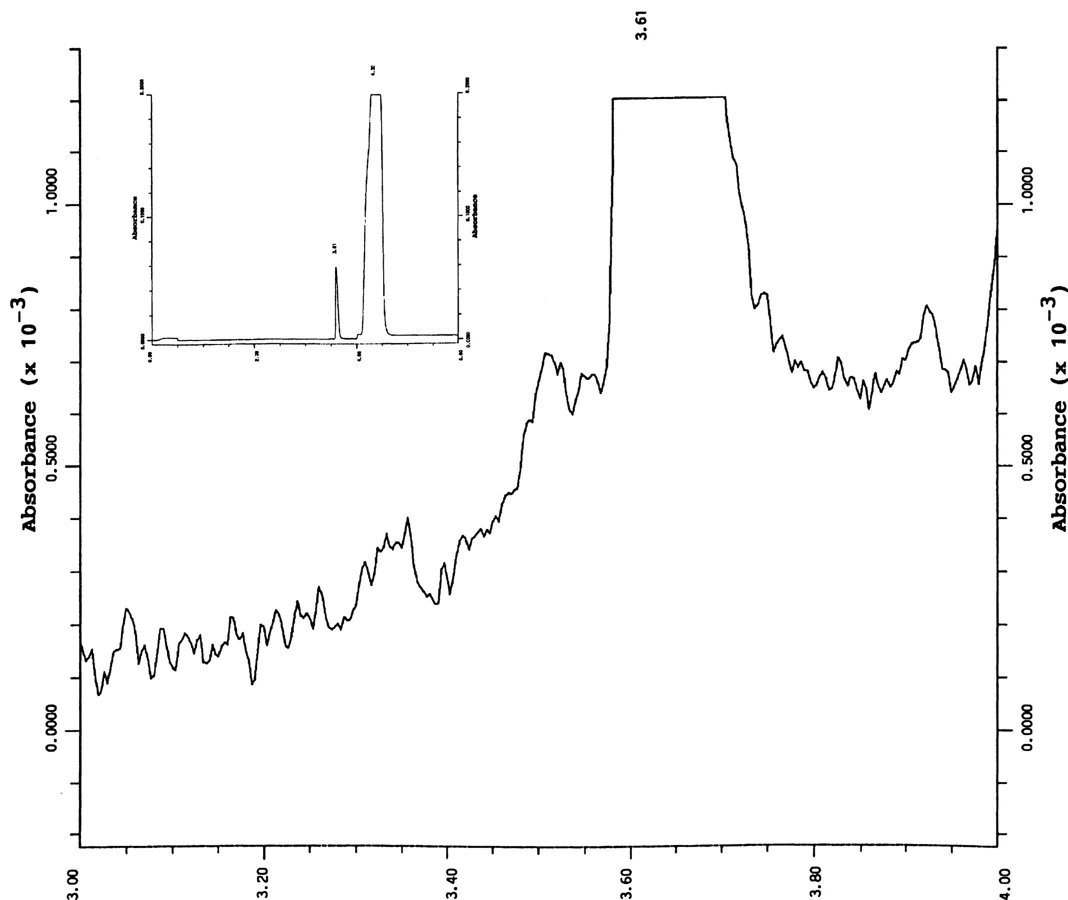

Fig. 1. Preliminary experiments: good peak shape, sufficient separation efficiency (five peaks/min) but insufficient sensitivity; signal-to-noise ratio about 300. Side compounds cannot be observed. Phosphate buffer pH 6.5, 60 mmol/l, additionally containing 250 mmol/l potassium sulfate. U=10 kV (115 μA), λ=214 nm, hydrodynamic injection 10 s, 34.5 mbar.

On the other hand, sample solution is sucked into the capillary by electroosmosis during FASI. Thus an undesirable plug of low conductivity is introduced. This plug consumes a considerable amount of the total field strength. Moreover, this plug is heated during the following separation, which can lead to precipitation of the analytes and blocking of the capillary. The volume of this plug linearly increases with injection time and voltage.

In addition to FASI, it was intended to improve sensitivity by optimising the sample pretreatment. In comparison to earlier experiments, the sample solution became about tenfold more concentrated of buserelin (Section 2.2).

However, using these concentrated samples, blockage of the capillary has been frequently observed. This was explained by precipitation of the main compound due to the very high concentrations achieved using FASI. It became necessary to reduce the injection time. A reduction of the separation voltage at the same time prevented these blockages.

This final method given in Section 2 (maximum sample concentration, medium FASI times) was preferred to the method outlined in Fig. 2 (low sample concentration, long FASI times). Using the final method, overall analysis time is shorter and the risk of sample depletion (compare Section 3.4.5) is reduced. Using the maximally concentrated sample solution, side compounds could be detected using hydrodynamic injection. However, the signal-to-

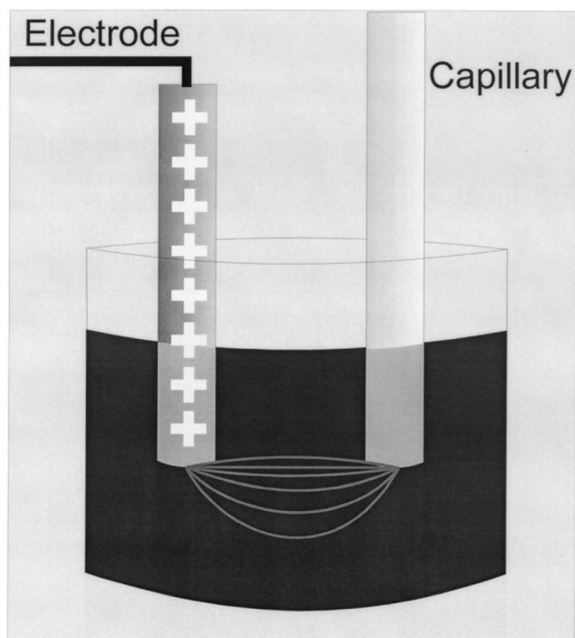

Fig. 2. Inhomogeneous field strength between electrode and capillary inlet.

noise ratio was still about fivefold better when FASI with 20 s injection time was used.

In this case the signal-to-noise ratio for the side compounds could not be further improved using longer injection times because of the limited solubility of the main compound. In general, using FASI, the maximum gain in sample concentration is limited by solubility.

3.3. Improvement of selectivity

After sufficient sensitivity had been obtained, selectivity has been optimised varying the buffer pH. Just the buffer constituents have been changed, the concentration of potassium sulfate was kept constant, as well as FASI injection conditions.

A buffer of pH 5.5 has been used for the electropherogram in Fig. 3. When the pH is further increased (up to pH 12), only poor separations are obtained. At these pH values the histidyl moiety is partly deprotonated. Probably the loss in charge leads to a loss in mobility differences and therefore a loss in selectivity. Using a pH of 12, a separation has been achieved (not shown). However, migration times were rather unstable, probably due to the absorbance of carbon dioxide.

When buffers with pH below 5.5 were used, resolution increased, mainly because of a decrease of the electroosmotic flow. No changes in selectivity have been observed. Using pH values of pH 3.5 or below, sufficient separation has been obtained. Now radiation-exposed implants could be readily distinguished from nonsterilised ones (Fig. 4).

3.4. Method validation

3.4.1. General considerations

The method developed so far (Fig. 4) seems to be able to answer the questions about the buserelin behaviour under radiation. In order to test if the method was really fit for this purpose, validation became necessary. Validation requirements for purity tests have been suggested by Altria and Rudd [17] (Table 1). Here, in addition, sample depletion has been investigated. A considerable amount of sample is injected every time FASI is used. However, this amount misses for the next injection. It should be investigated, whether or not this effect is of importance.

3.4.2. Validation of precision

Two additional compounds with considerable peak areas were found in radiation-exposed implants. Both migrate past the buserelin main peak (migration time, $t_m = 19.73$ and 20.49 min in Fig. 4B). The amount of these compounds is estimated using relative peak areas. The reproducibility of this estimation has been studied. Six high-dose exposed implants were prepared independently and each injected twice. The peak areas of the two additional compounds were evaluated together and compared to the total peak area. A mean of 1.745 area% has been obtained with a standard deviation (S.D.) of 0.184 area%. Thus the relative standard deviation (R.S.D.) of this relative area determination equals 10.5%.

This precision is good enough to compare differently treated implants. However, precise quantitative results are not possible with this so far developed method. The actual sample concentration is in the range of the limit of quantitation.

Usually much better precision data is possible in CE. However, here sample preparation is another

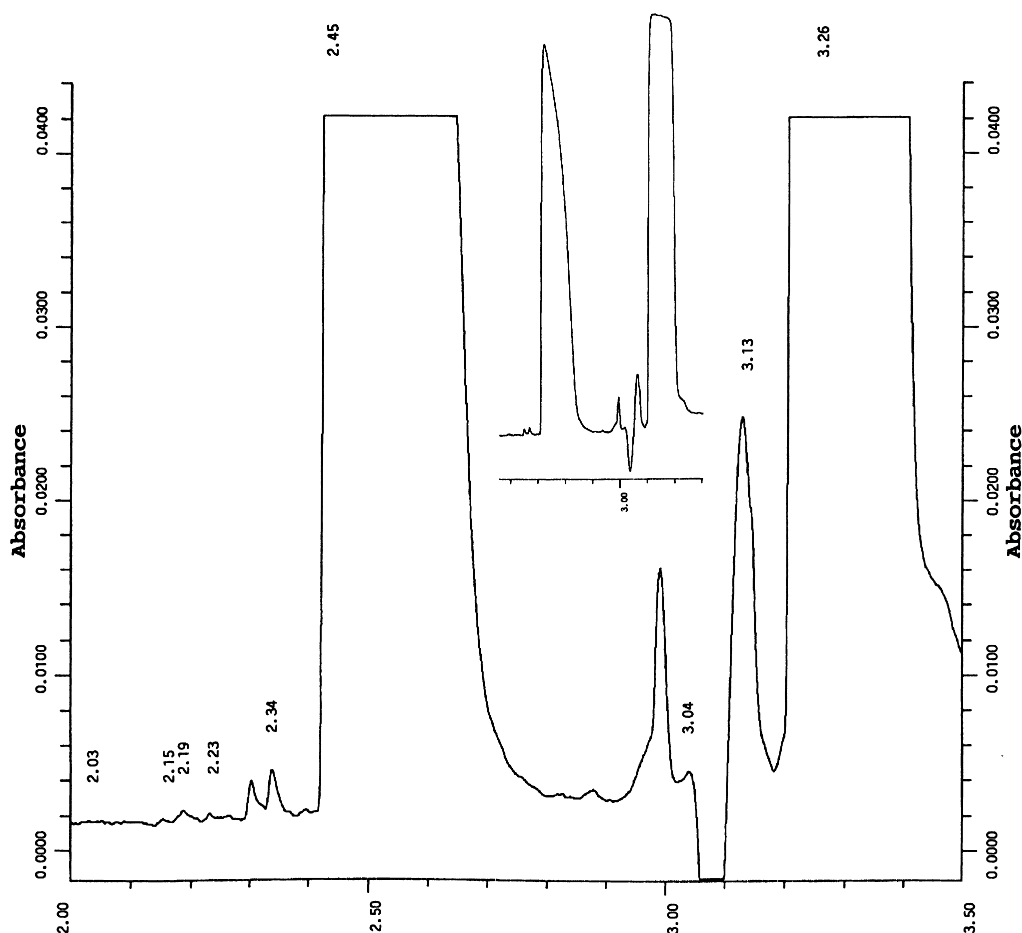

Fig. 3. Field-amplified sample injection (FASI). The signal-to-noise ratio was improved by a factor of 50 compared to the initial experiments (see Fig. 1). $U=15$ kV (200 μA), $\lambda=214$ nm. Field-amplified electrokinetic injection 300 s, 1 kV. Same sample pretreatment used as in Fig. 1.

considerable error source. Before attempts are made to improve precision, the importance of the different error sources has been estimated using the law of error propagation.

Six samples have been injected twice each. The difference between the two subsequent injections does not depend on the sample preparation. As long as area% is compared, sample depletion by FASI does not play a role (compare Section 3.4.5). The S.D. of single measurements can be estimated from the S.D. of differences according to Eq. (1).

$$\text{S.D.}(\Delta x) = \sqrt{2} \times \text{S.D.}(x) \quad (1)$$

The S.D. of the six obtained differences, S.D.(Δx), has been estimated as 0.1664 area%. Thus the error caused by CE, S.D._{CE}, equals 0.1177 area%, corresponding to a R.S.D. of 6.7%.

Now the error due to other sources $\text{S.D.}_{\text{rest}}$ can be calculated using Eq. (2):

$$\text{S.D.}_{\text{tot}}^2 = \text{S.D.}_{\text{CE}}^2 + \text{S.D.}_{\text{rest}}^2 \quad (2)$$

Here S.D._{tot} represents the total error of 0.184 area% (R.S.D.=10.5%). $\text{S.D.}_{\text{rest}}$ is estimated as 0.1107 area% (R.S.D.=6.3%).

Both sample preparation and CE are major error sources. The CE error is due to the relatively low signal-to-noise ratio and the subsequent difficulties

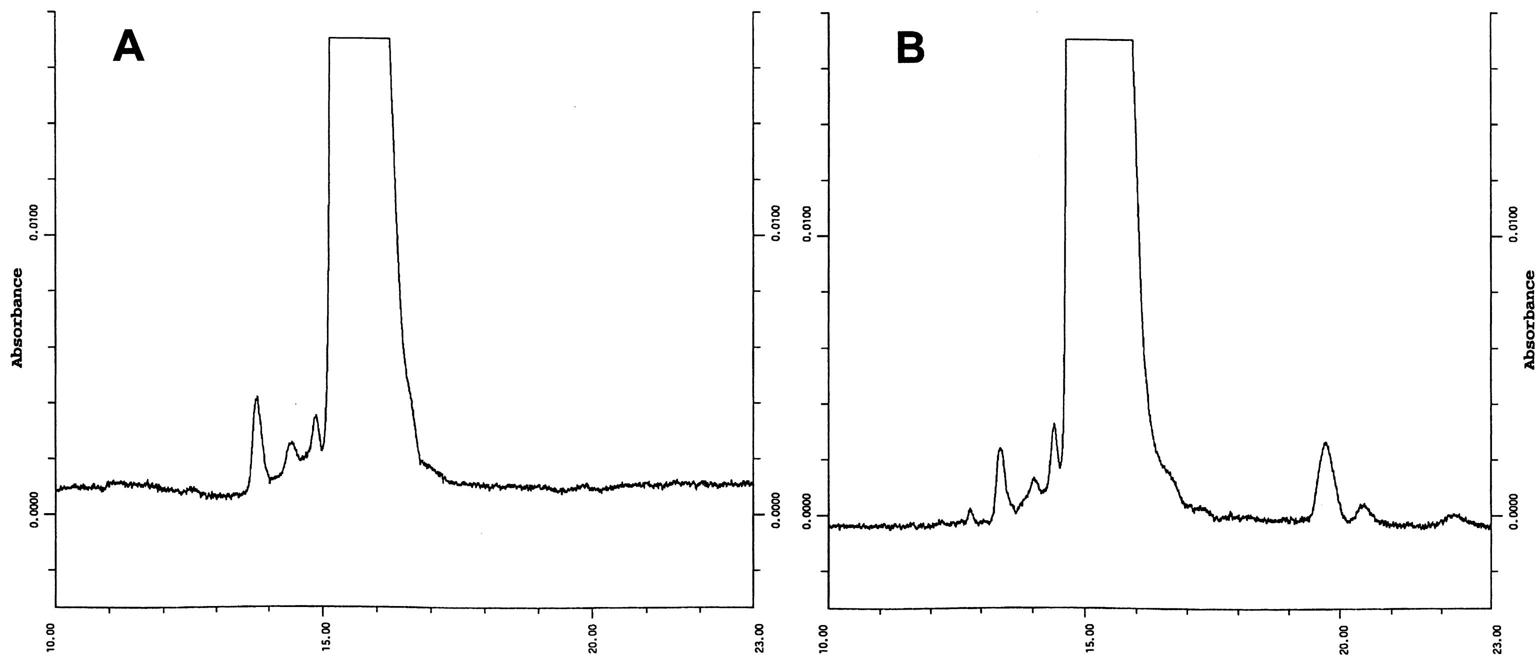

Fig. 4. Separation of buserelin from side compounds at pH 3.0; improved sample pretreatment (Section 2.2). $U=8$ kV (100 μA), field-amplified electrokinetic injection 20 s, 1 kV. (A) Not ray-treated (area% of side compounds≈0.2%); (B) implant exposed to unusually high dose of γ-radiation (area% of side compounds≈2.0%).

Table 1
Check list for validations of purity determinations (compare Ref. [17])

Validation requirements	Confirmed by:
Accuracy	HPLC cross validation (not shown)
Precision	Section 3.4.2
Linearity	Area% (linear to injection time (data not shown)
Sensitivity	Optimised by method development
Selectivity	Section 3.4.3
Limit of detection	$<$0.1 Area%
Limit of quantitation	Section 3.4.2; only semiquantitative results
Solution stability	Section 3.4.4
Robustness	Not tested (too expensive; accuracy has been proved)
Here, in addition: sample depletion	Section 3.4.5

of peak integration. The results could get better using improved detectors [18] and integration software [19].

However, it is very difficult to reduce the considerable amount of other error sources. $S.D._{rest}$ is partly caused by the heterogeneity in the sample implants, possibly due to slightly varying radiation or storage conditions. Furthermore, there are additional error sources due to the sample preparation which are hard to overcome. Volume dosage could be improved, but after centrifuging varying amounts of sample remain within the implants. It seems very difficult to quantitatively extract the sample compounds into the supernatant without too much dilution. Moreover, the sample decay observed in Section 3.4.4 also begins to take place during sample preparation. Therefore it was decided to use the actual method keeping in mind this limitation.

3.4.3. Validation of selectivity

CE is often a compromise between sensitivity and selectivity. If maximum selectivity is desired, separation efficiency should be optimized. This means that the injected sample amount should not be too high to avoid overload effects and subsequent peak broadening.

However, the method under investigation has been optimized for sensitivity in order to detect minor compounds. The main peak of buserelin is already overloaded, which can be seen from the peak width. In order to see if additional compounds are hidden under the main peak, the injected sample amount has been subsequently reduced. Using an injection time of 2 s, the peak width of buserelin is about the same as of the minor compounds (Fig. 5). At the same time the signal-to-noise ratio gets worse. Two additional compounds can be observed. However, these occur in sterilized and nonsterilised samples. Thus these compounds are not relevant to understand what happens when radiation is applied. The selectivity of the method is sufficient. The injection time was not reduced in order to keep the best signal-to-noise ratio possible.

3.4.4. Validation of sample stability

Is it necessary to freshly prepare every sample before analysis or are the samples stable such that it is possible to work up a number of samples and analyse them subsequently using an autosampler? The latter would make work much easier, but this is only possible when the samples do not significantly change during storage.

A decrease of one compound ($t_m = 19.5$ min in Fig. 4B) has been observed in several experiments. In order to study this systematically, nine subsequent injections were done from one sample. The relative area of this compound reduced to less than 50% during the storage time in the autosampler (Fig. 6). Thus it is necessary to prepare every sample freshly and analyse it immediately after preparation.

3.4.5. Validation of sample depletion

This issue was investigated in addition to the parameters suggested in Ref. [17], because a considerable amount of sample is injected every time FASI is used. This amount misses for the next injection. It should be investigated, whether this effect is significant.

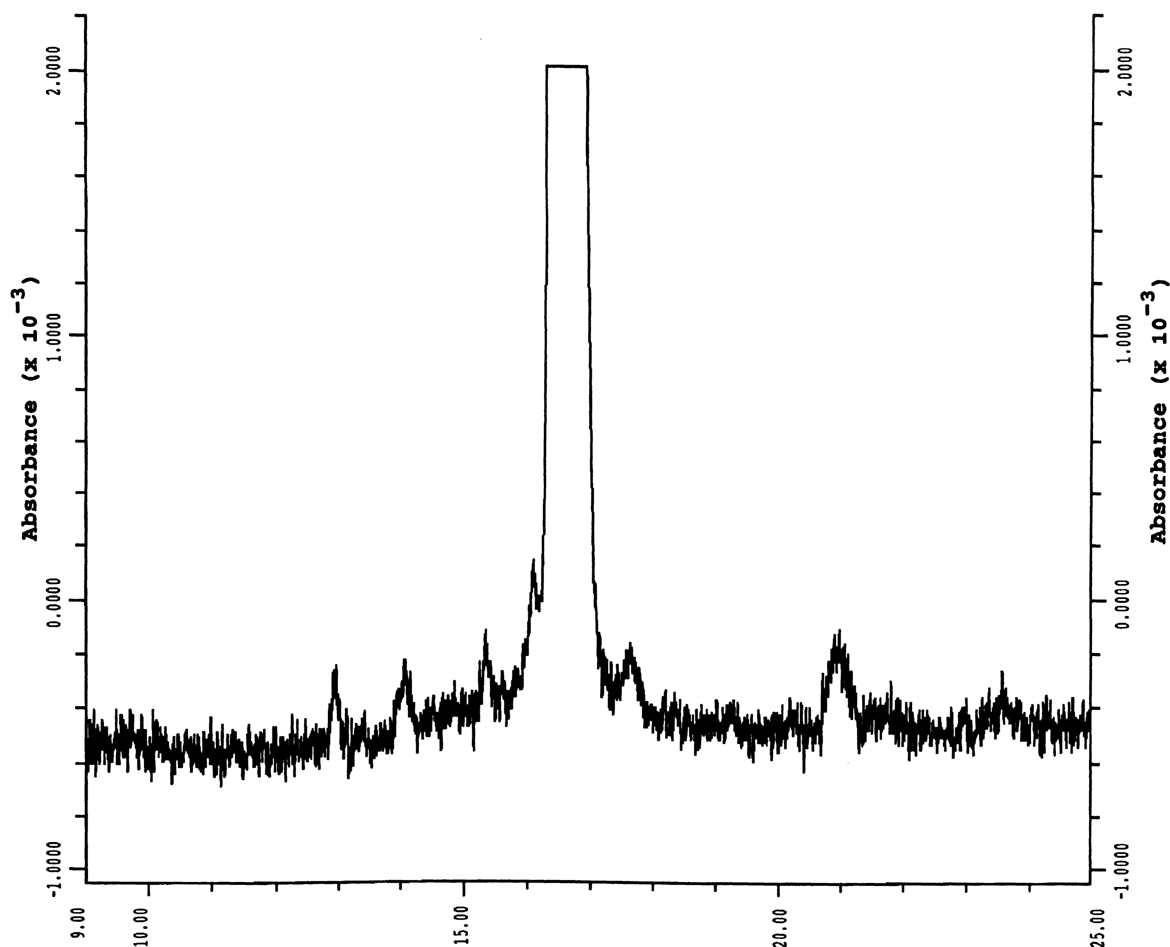

Fig. 5. Two additional peaks are detected when the injection time is reduced to 2 s (compare Fig. 4B). However, the corresponding substances are found as well in treated as in nontreated material.

In order to test this, 12 subsequent injections were done from one sample. This time not the relative peak areas were considered but the absolute peak area of the buserelin main peak as a measure for the material injected. As can be seen from Fig. 7, sample depletion causes significant effects when FASI is used. Absolute peak areas can only be precisely determined when every sample is used for only one injection.

However, the sample is not completely used up by only one FASI injection. It is possible to do a second injection to control the result of the first, but relative instead of absolute peak areas must be used. In our investigations no significant changes of relative peak areas have been observed due to depletion. However, it is advised to validate the constancy of relative peak areas for each new FASI method, if several injections are done from one vial.

3.5. Sample instability: subsequent reactions

During validation instability of one of the compounds has been observed (Section 3.4.4). It is very interesting to study the reaction that causes this instability. A first idea how to do this could be preparative CE. This option has been reported to allow, e.g., Edman sequencing or MS analysis after discontinuous fraction collection [4]. In our investigations the main compound could also be detected

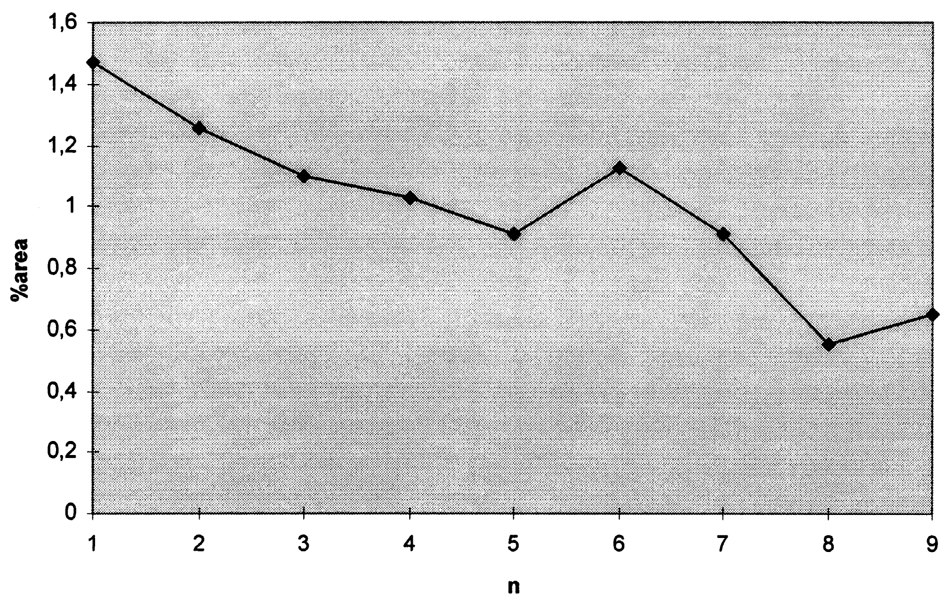

Fig. 6. Subsequent field-amplified sample injection from one sample: area% of one compound ($t_m = 19.5$ min in Fig. 4B) decreases due to instability.

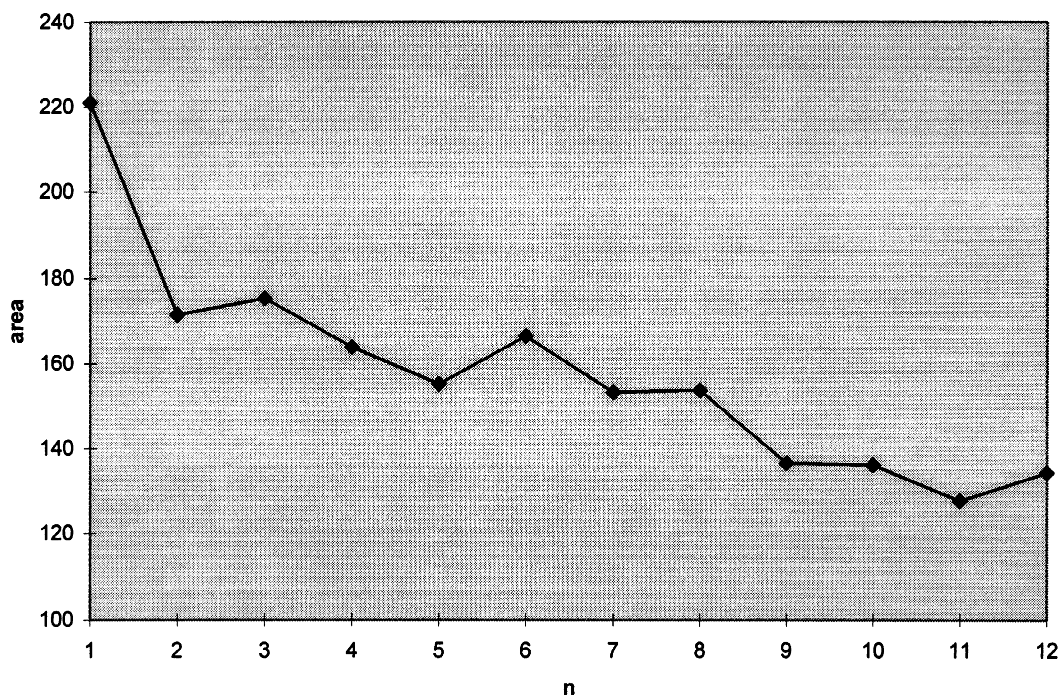

Fig. 7. Subsequent field-amplified sample injection from one sample: area of main peak decreases due to depletion.

in collected fractions when a 75 μm capillary has been used.

However, no side compounds could be found using preparative CE. This is because of a dilution effect of about 1:300. Only very small amounts (injected sample volume is in the nl range) are injected in CE, but a volume of at least 20 μl in the collecting vial is necessary to guarantee a stable current. In order to avoid this dilution problem, on-capillary observation of the radiation product decay has been introduced.

Therefore, at first a usual CE separation is carried through. Peaks are detected when the compounds pass the detection window (Fig. 8A, Fig. 9). However, the separation is continued after the compounds have already been detected (Fig. 8B), until the main compound buserelin passes the capillary outlet (Fig. 8C). The time when this happens can be calculated from the migration times, the effective and the total length of the capillary (see Section 2). Now the voltage is reversed and the side compounds, which are still in the capillary, migrate backwards. As the substances pass the detector, complete separation from buserelin is confirmed (Fig. 8D, Fig. 10).

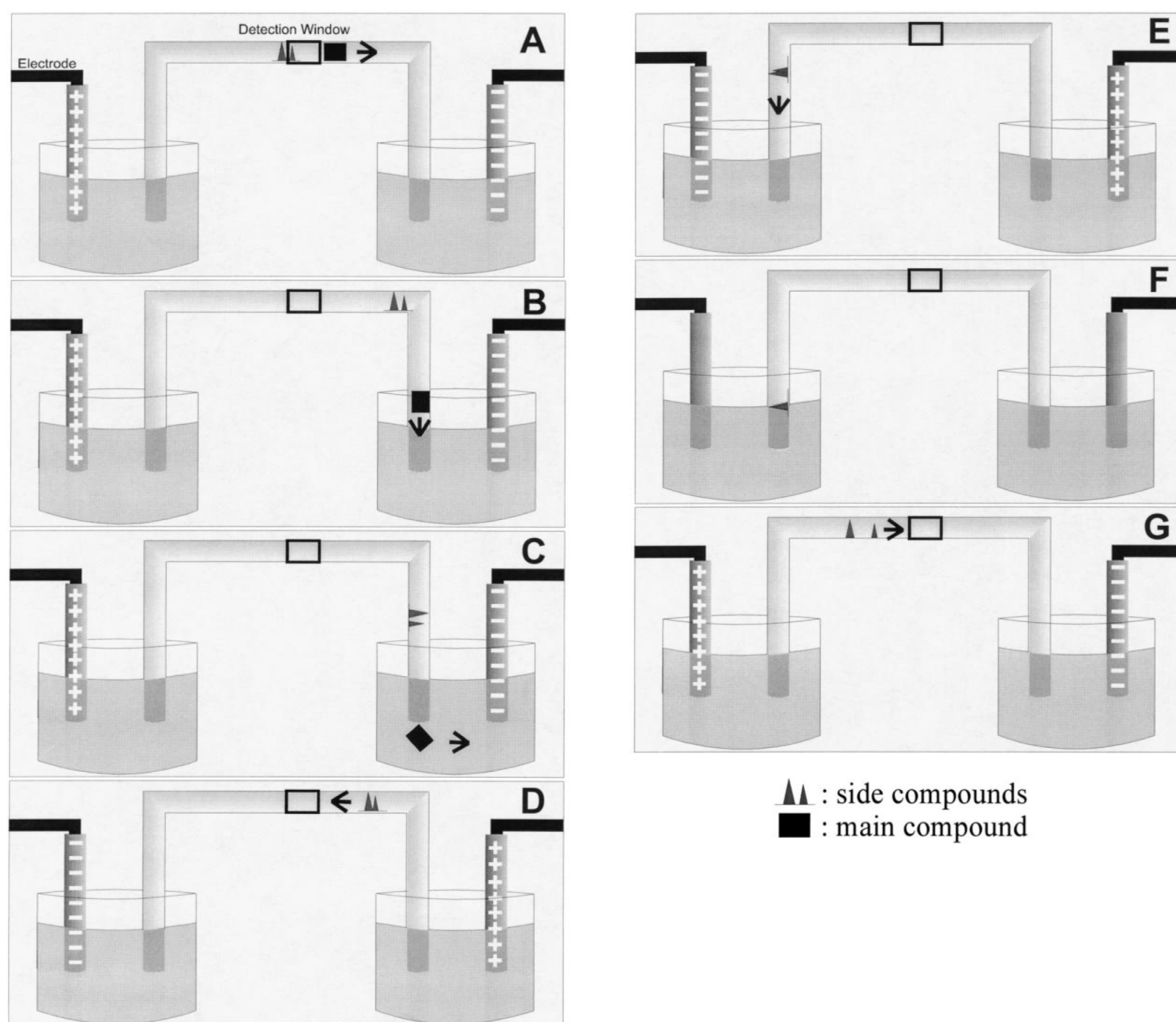

Fig. 8. Concept of on-line observation of radiation product decay.

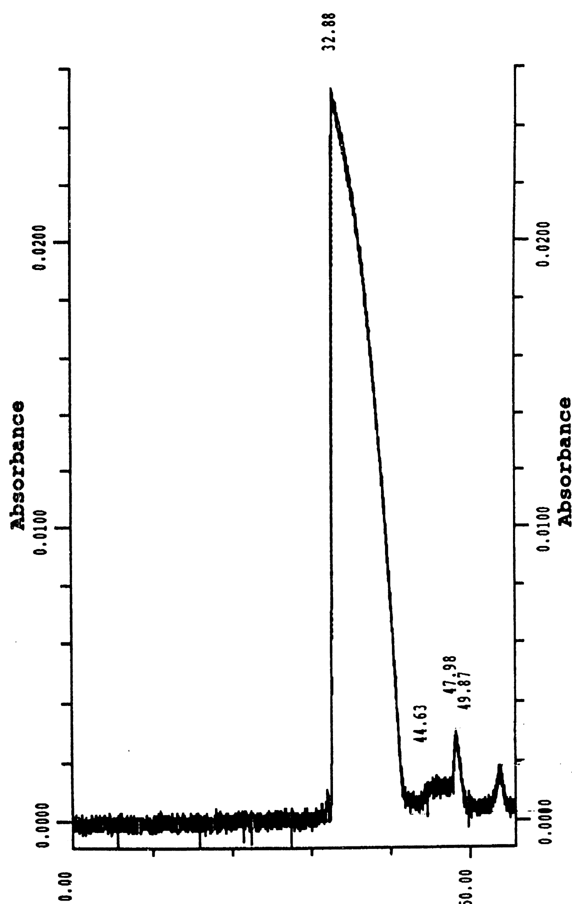

Fig. 9. CE separation as usual (Fig. 8A). The time to switch polarity is calculated from the beginning of the first side compound peak (here: 44.63 min).

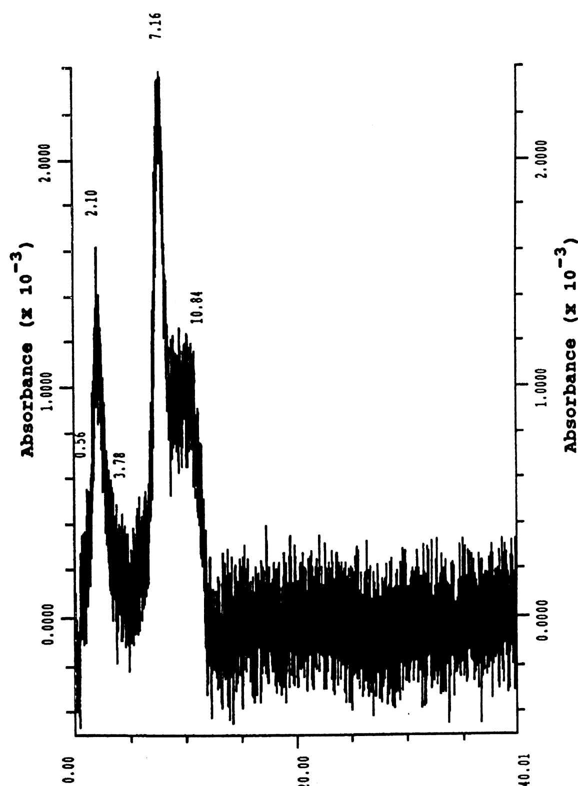

Fig. 10. Reversed voltage; radiation products migrate backwards in reversed order. Compounds eluting from 44.63 to 55 min in Fig. 9 now elute from 0 to 11 min, e.g., the peak 47.98 in Fig. 9 corresponds to the peak at 7.16 min. Complete separation of buserelin is confirmed (see also Fig. 8D).

During their way back the various compounds come together to one zone again: substances that have faster migrated during the first separation also faster migrate backwards (Fig. 8E). The reunited zone is stored for several hours (Fig. 8F). Then the polarity is switched again: subsequently formed substances can be separated. The peaks are broader from the diffusion during the storage process in Fig. 8F, but separation is still good (Fig. 8G, Fig. 11).

At least two, possibly four compounds are formed during the decay of the primary γ-ray products. The corresponding signals however have also been found in nonsterilised samples that have been stored for more than half a year. Thus these secondary products should not mean any additional risk for the patients' health.

4. Conclusions

Buserelin is stable within its formulation when exposed to γ-radiation. Additional side compounds are found, but area% of side compounds is still only about 2%, even if unusually high radiation doses are applied. The reactions of decay of these compounds can be characterised. All finally formed substances have also been found in untreated implants after long-time storage. Radiation-exposed implants can easily be distinguished from nonsterilised formulations.

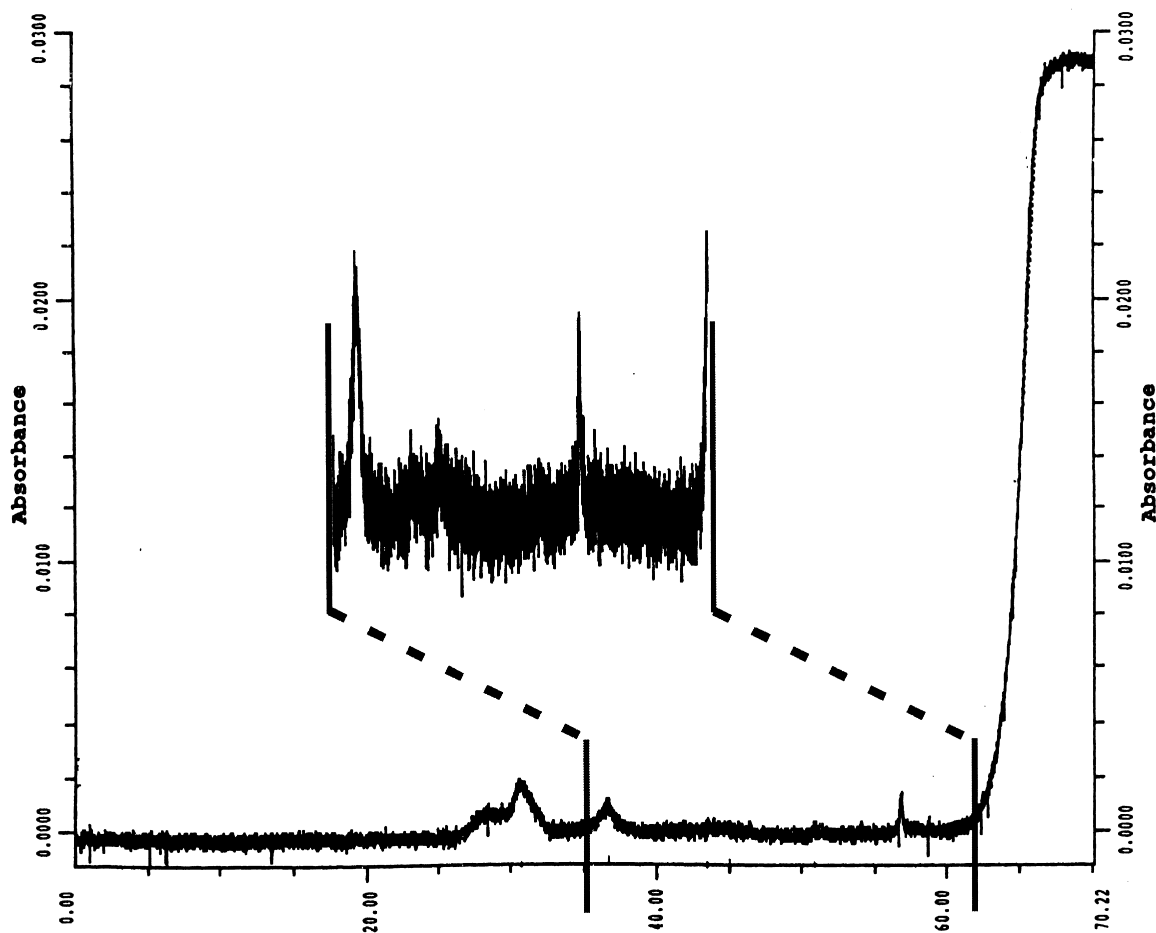

Fig. 11. After several hours this zone is separated again using normal polarity. Secondary products of radiation can be characterised (Fig. 8G). The compounds that elute from 44.63 to 55 min in Fig. 9 now elute from 26 to 38 min. In addition to that, at least one compound at 54 min is detected, and maybe two more at about 40 min. The signal after 60 min corresponds to the sample solvent DMF.

FASI has been successfully applied to improve sensitivity. This technique is best when relatively low voltage (e.g. 1 kV) and long injection times (e.g. 20 s) are used. A validation concept for this mode of operation is outlined for the first time. Sample depletion causes significant effects. Multiple injections out of one vial must be interpreted with care.

A within-capillary stability test has been successfully introduced. The major shortcoming of preparative CE, dilution in the collecting vials, has been avoided by this method.

References

[1] E. Mutschler (Ed.), Arzneimittelwirkungen, sixth ed., Wissenschaftl. Verlagsges. Stuttgart, 1991.
[2] P.G. Righetti, C. Gelfi, M. Perego, A. Stoyanov, A. Bossi, Electrophoresis 18 (1997) 2145.
[3] A. Cifuentes, H. Poppe, Electrophoresis 18 (1997) 2362.
[4] I. Messana, D.V. Rossetti, L. Cassiano, F. Misiti, B. Giardina, M. Castagnola, J. Chromatogr. B 699 (1997) 149.
[5] H. Wätzig, C. Dette, Pharmazie 49 (1994) 83.
[6] C. Dette, H. Wätzig, H. Uhl, Pharmazie 48 (1993) 276.
[7] J.S. Green, J.W. Jorgenson, J. Chromatogr. 478 (1989) 63.
[8] R.-L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.

[9] R.-L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 153.
[10] C.-X. Zhang, Y. Aebi, W. Thormann, Clin. Chem. 42 (1996) 1805.
[11] C.-X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[12] H. Wätzig, C. Dette, Pharmazie 48 (1993) 75.
[13] M. Unger, J. Stöckigt, J. Chromatogr. A 791 (1997) 323.
[14] F. Tagliaro, G. Manetto, S. Bellini, D. Scarcella, F.P. Smith, M. Mango, Electrophoresis 19 (1998) 42.
[15] C.-X. Zhang, W. Thormann, Anal. Chem. 70 (1998) 540.
[16] Y. Mechref, Z. El Rassi, Electrophoresis 18 (1997) 220.
[17] K.D. Altria, D.R. Rudd, Chromatographia 41 (1995) 325.
[18] A. Kunkel, M. Degenhardt, B. Schirm, H. Wätzig, J. Chromatogr. A 768 (1997) 17.
[19] B. Schirm, H. Wätzig, Chromatographia, in press.
[20] S.A.J. Coolen, F.M. Everaerts, F.A. Huf, J. Chromatogr. A 788 (1997) 95.