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A Multi-Analytical Technique Approach to the Dating of Writing Inks.

1. INTRODUCTION

Relative Dating

The present method of determining whether or not a document is as old as it is claimed to be, by comparison with a reference collection of standards was first described by Hofmann in Switzerland [1], using a combination of chemical and chromatographic tests. This approach was developed in the United States by Brunelle [2] with the introduction of a comprehensive ink reference collection, presently maintained by the Secret Service in Washington DC.

The relative dating of an ink is approached by comparing the properties of a questioned sample against a library of samples of known age and origin. As described above, this is usually achieved by the comparison of the thin layer chromatographic pattern of the ink under investigation with that of the known sample. If the two inks are found to match, the manufacturers files are consulted to determine when the ink was first manufactured. While this approach can be useful in determining the authenticity of a document it has limitations. It is only possible to say that a document could not have been written before a certain date. It is not possible to determine the actual date that the document was prepared.

In some cases, inks can be dated by the identification of tags or other unique components added by the manufacturer. This also has limitations since only a few inks actually contain these tags.

Aging of Inks

The determination of the actual age of a document by examination of the ink has received a great deal of interest over the last decade or so. Although some progress has been made in this field, the solution still eludes researchers and document examiners alike. The ability to determine when a document was written would rate as one of the major breakthroughs in forensic science, having a significant impact on the detection of all kinds of fraud. This would result in a corresponding financial benefit to both State and Federal administrations.

Several analytical approaches have been tried by different workers, to determine the age of an ink. The most successful of these include; the effect of time on the relative solubility of the ink in different solvents; changes in the relative concentrations of dyestuffs and solvents in the ink as determined by chromatographic and/or spectral methods and; changes in the infra-red absorbance spectrum of the ink over time.

The determination of the absolute age of a document by examination of the ink has been the subject of several studies during the last 20 years. However, in contrast to the numerous articles in the literature for the differentiation of inks, only a few researchers have published procedures for dating inks. As early as 1937, chemical tests on iron gallotannate inks were described as having potential for the estimation of the age of a document [3]. Later in 1959, Kikuchi [4] provided the basis for the solvent extraction techniques in use today, for estimating the age of writing inks. This work involved the measurement of the time taken for blue-black inks to disperse when solvents were applied to the ink on paper. Kikuchi observed that older inks dispersed more slowly than newer inks. Much later, Cantu [5] used the principles applied by Kikuchi to develop a procedure for estimating the age of ballpoint ink. He found that a relationship existed between the age of ballpoint ink and the rate at which the ink could be extracted from paper using low and medium polarity solvents such as toluene, isopropanol and butanol. The longer the ink had been applied to the paper the drier it became and the slower it extracted into solvents. If known dated inks covering a span of years are present on the questioned document, for example in a ledger, then it is possible to closely estimate when a questioned entry was written by comparison of the extraction rates. This approach is however, only useful if the known and dated inks are of the same ink formulation and that they are on the same paper, thereby eliminating storage condition variables. In addition, by determining the amount of dye extracted into a given solvent at two or more times, these amounts can be compared as ratios. In essence this approach, known as the R-Ratio method, allows a particular ink to be described numerically by virtue of these amount ratios. The concentrations of ink extracted by the solvent were determined by means of their absorbance in the UV/Vis spectrum by means of a densitometer after application of a known volume of the extract to a TLC plate. If the ink contains fluorescent compounds, the amount of fluorescence in the extract can also be monitored. The use of a fluorescence spectrophotometer improves the sensitivity of the analysis, but is susceptible to interference from fluorescent compounds in the paper.

The use of sequential solvent extraction techniques for ballpoint pen inks has also been described. Solvents of different polarities were used to sequentially extract an ink sample. The amount of ink extracted by the first solvent can be expressed as a percentage of the total amount of ink extracted by all of the solvents. A combination of toluene as the first extraction solvent followed by benzl alcohol was reported to give optimal results for ball pen inks.

In 1987, Brunelle [2] reported a single solvent extraction procedure to estimate the age of ballpoint inks. Like Cantu's work, this procedure also involved the extraction of inks with weakly polar solvents, spotting the extract onto a TLC plate and measuring the amount of ink extracted by the use of a densitometer. Age of ink was estimated by comparison with known dated inks. The main limitation of this technique is that it is mass dependant and requires that equal samples of questioned and known ink be removed for analysis. Brunelle later used TLC to separate his ink extracts with a mobile phase of ethyl acetate: ethanol: water (75:35:30). The relative concentrations of different dye components in the extract were compared [6]. This represents one of the most extensive pieces of work available on the age determination of inks. The calculated ratios of the relative concentrations of the dyes are independent of the amount of ink taken for analysis. The effect of using different extraction solvents, the effect of the paper and the reproducibility of the technique were determined.

Two other analytical approaches to the aging of inks deserve mention in this review of the literature;

In 1985, Stewart [7] reported a gas chromatography procedure for comparing the relative age of ballpoint inks. This work was based on the gradual disappearance with time of the solvents contained in ball point inks. It was reported that these solvents remain in dried ink on paper for up to one year and longer for some ballpoint inks. This technique involved extraction of dried inks on paper with strong solvents such as pyridine and then comparison of the relative concentrations of vehicle by gas chromatography.

Humecki reported another technique in 1985 for measuring the relative age of ballpoint inks using Fourier Transform Infrared spectroscopy with a microscope attachment [8]. Changes in the hydroxyl, methyl and carbonyl absorption bands were seen as the ink aged. He found a decrease in the ratio of hydroxyl to methyl bands in ballpoint ink up to 22 years. This work was however limited to only one ballpoint ink formulation and has not been verified. The use of FTIR spectroscopy for the examination of inks was also reported by Becker and Brunelle [9]. This technique may become of greater importance for ink analysis, given the recent introduction of a instruments which combine TLC and FTIR [10].

Future Trends

Thin layer chromatography is a well established technique for the comparison of inks. TLC procedures are rapid and sensitive and have been optimized over the years by document examiners and ink chemists. Thin layer chromatography therefore still has a very important role to play in the routine examination of inks. There is however presently a great deal of research and development work being performed involving the evaluation of more sophisticated and sensitive instrumentation for ink analysis. Of these, HPLC has the sensitivity and improved resolution to make it an attractive technique for the examination of inks which cannot be distinguished by TLC [11].

Capillary electrophoresis [12,13] is a relatively new procedure which has been introduced as a successor to traditional paper and gel electrophoresis. The technique is quickly generating a great deal of interest particularly in the field of forensic serology, because of its improved sensitivity, resolution and speed. Preliminary work has also been described using capillary electrophoresis for the separation of inks [14].

The use of gas chromatography for the examination of the volatile components of inks has been described above. However the interfacing of gas chromatographs with Fourier Transform infra red spectrometers [15] and mass spectrometers [16] may be useful not only for the comparison of inks but also for the identification of the ink components. Information on the latter is not usually available to the analyst, because of the proprietary nature of the inks.

In recent years, a great deal of research has been carried out on the use of supercritical fluids [17,18] for extraction and chromatographic purposes. Basically, under the correct conditions of temperature and pressure, the eutectic point, a gas such as carbon dioxide, has the properties of both a gas and a liquid. This supercritical fluid can be used for "solvent" extractions but with the advantage that at room temperature it evaporates leaving no solvent residue. Also since the fluid has both liquid and gaseous properties it can be used as the mobile phase for chromatographic separations, being compatible with both HPLC and GC detectors. Supercritical fluids are considered to have great importance in the chromatography of the future. Ink samples can also be extracted from their paper medium by the liquid carbon dioxide. The increasing addition of methanol to this supercritical extraction fluid gives us the ability to gradually increase the polarity of the extracting solvent. This technique has potential for the dating of inks as follows.

The applicability of "state of the art" chromatographic and spectroscopic techniques to the examination of inks is gradually being realized. These techniques will undoubtedly have a future role to play in the determination of the age of an ink. In addition, the increased sensitivity and resolution of these techniques gives the examiner a greater degree of certainty concerning the comparison of two samples. This may ultimately result in greater evidential value being placed on this type of analysis.

2. AIM OF PROJECT

The aim of this project was to determine the age of a document by extraction and subsequent analysis of ink. The analytical aspect of the work was relatively straight forward, employing techniques such as gas chromatography, FTIR and HPLC which have previously been used by this laboratory for the examination of writing inks. The novelty of the approach and the part which resulted in the most problems, was the use of supercritical fluids for the preferential extraction of inks from the paper.

3. METHODS

3.1 Initial Conditions

An inked line was cut from the paper and placed in the extraction vessel of a Suprex SFE 50 extraction unit. Extraction of the ink was achieved with supercritical carbon dioxide, with the oven temperature programmed isothermally at 65°C for 15 minutes, at a pressure of 100 atmospheres. The extractor was held five minutes in the static mode and switched for ten minutes to the dynamic mode. The eluent was collected in a 10 x 75 millimeter glass tube containing 500 microliters of methanol. This methanolic extract was then evaporated to dryness under nitrogen before subsequent analysis. The dried sample was reconstituted to 50 microliters in methanol and aliquots were used for analysis by GC-MS, FTIR and HPLC.

3.2 Difficulties encountered

The difficulty has been with the incorporation of an organic solvent into the supercritical fluid in order to make this sufficiently polar as to allow extraction of the ink components. To this end we have made the following observations:

a). The volatile components of the inks can be extracted directly with supercritical carbon dioxide, allowing short term determination of age based on the evaporation of these solvents.

b). Other UV absorbing components can be extracted with the incorporation of 10% methanol into the supercritical fluid. This however does not remove all of the ink components from the paper and has been unsuccessful in distinguishing between inks of different ages.

c). Addition of butanol up to a concentration of 4% in the CO_2 improved the extraction of ink components from the paper.

d). Incorporation of pyridine into the supercritical fluid resulted in complete extraction of the ink from the paper. Problems were encountered in dispersing pyridine into the carbon dioxide and delivering it in a reproducible manner to

> the sample. This problem was circumvented by the use of a hydromatrix in the sample cell. The hydromatrix absorbs any moisture present in the extraction vessel and seems to have prevented problems associated with inconsistent extraction.

At this point we had the capability of extracting and differentiating inks. No one procedure however allowed us to determine the age of the ink. However, after consultation with Dr Cantu of the US Secret Service we designed experiments based on his work with the differential extraction of inks with solvents of different polarities. Since supercritical carbon dioxide incorporating pyridine appeared to be our "all extracting solvent", compared the extraction of individual ink components by this solvent with the degree of extraction of the same compounds by weaker solvents such as CO_2 , and CO_2 incorporating methanol or butanol. Using this approach, we perfected the technique of reproducibly extracting ink from paper using supercritical fluids.

The procedure was improved significantly by the realization that the effective strength of the solvent could be modified by changes in operating pressure. This approach circumvented the need to change the solvent composition in order to change solvent strength, thereby simplifying the technique and greatly reducing the time of analysis.

3.3 Optimum Extraction Method

The optimum procedure for the extraction of ink samples was as follows:

1. Mobile phase : 10% Methanol in Carbon Dioxide.
Temperature : Isothermal at 65°C
Pressure : 250 atm Static for 1minute
250 atm dynamic for 5 minutes.

Followed by:

2. Mobile phase : 10% Methanol in Carbon Dioxide.
Temperature : Isothermal at 65°C
Pressure : 300 atm Static for 1minute
300 atm dynamic for 5 minutes.

Followed by:

3. Mobile phase : 10% Methanol in Carbon Dioxide.
Temperature : Isothermal at 65°C
Pressure : 350 atm Static for 1minute
350 atm dynamic for 5 minutes.

In each case, the eluent was collected in a 10 x 75 millimeter glass tube containing 500 microliters of methanol. This methanolic extract was then evaporated to dryness under nitrogen before subsequent analysis.

3.3.1 Explanation

The ink sample is placed in a sample cell within the supercritical fluid extractor. The cell is flooded with the mobile phase at 65^{o}C and 250 atmospheres pressure. Extraction of the ink from the paper is allowed to take place for 1 minute (static mode). The mobile phase is then allowed to pass through the sample and is collected in a sample tube for a further 5 minutes (dynamic mode).

Without removing the sample from the extractor, the process is repeated at 300 and again at 350 atmospheres. As the pressure is increased so is the amount of sample extracted. This process therefore essentially gives us three extractions of the same sample, differing in the strength of the extraction solvent.

3.4 Analysis

The extracted ink samples were compared by analysis with Fourier Transform Infra red spectroscopy (FTIR), Gas Chromatography-Mass spectrometry (GC-MS) and High Performance Liquid Chromatography (HPLC).

3.4.1 FTIR

A Mattson, Galaxy 4020 bench FTIR spectrophotometer coupled with a Spectra-Tech IR plan microscope was used to obtain IR spectra of the ink samples. Extracts were evaporated to dryness onto a BaF_2 salt window (Spectra-Tech Inc.) and placed under the IR microscope. FTIR spectra were obtained directly from the microscope stage by scanning 128 times at a resolution of 4.0cm^{-1}. IR spectra were obtained over the range 900 to 4000cm^{-1}., with a mercury, cadmium tellurium (MCT) detector. Data manipulation was achieved with a Leading Edge D3/25 PC using the

Mattson "First" software. particular reference was made to the hydroxyl, methyl and carbonyl groups.

3.4.2 GC-MS

A Finnegan 4510 GC-MS was used with helium carrier gas at 1ml/min and using a temperature program of 50°C to 290°C at 10°C/min. Separation was achieved using a 15m DB-1 column. Full electron impact mass spectra of eluting peaks were obtained .

3.4.3 HPLC

A Perkin Elmer Series 3B pump was used to deliver mobile phase at 1.5ml/min to a 25cm x 4.5mm ID microbondapak C18 column (Waters). The mobile phase consisted of 80% Acetonitrile : 20% 0.002M Heptane Sulfonic acid to which 2ml of glacial acetic acid was added. Detection of eluting compounds was achieved with a Spectra Physics Focus detection system and integration and data manipulation with an IBM PS/2 computer. Samples were injected onto the system via a Rheodyne injection system incorporating a 20ul loop. The chromatographic profile of each ink sample was obtained at all wavelengths in the ultraviolet and visible regions.Estimation of peak purity and comparison of peaks in different chromatograms having similar retention times was achieved by examination of the absorbance spectrum of each peak together with its first and second derivatives.

4. RESULTS and DISCUSSION

FTIR examination was disappointing showing no significant differences between extracts of the same ink. The use of FTIR for this purpose was therefore discontinued.

GC-MS showed some promising results, allowing not only the quantitation of volatile solvents in the extracts but also structural identification of certain ink components. The only limitation of this particular approach was the lack of sensitivity of this particular mass spectrometer (50-100ng/ml), thereby necessitating the use of large sample sizes.

Liquid chromatographic analysis of supercritical fluid extracts resulted in the greatest amount and most useful information. Extracts were evaporated to dryness under a stream of nitrogen, redissolved in 100ul of HPLC mobile phase and 20ul injected onto the instrument. Figures 1, represents a chromatogram of an extract of a 1 month old ball pen ink sample. The ink was extracted at 250 atmospheres as described above. The chromatogram shows 5 wavelengths superimposed on each other. Figure two shows the same sample subsequently extracted at 300 atmospheres pressure. Much less ink is present in the extract indicating that the bulk of the sample was extracted in the first extraction at 250atm. Analysis of the third extraction of this ink sample ie at 350atm yielded no useful chromatographic information indicating that all of the ink components had been removed from the paper by the initial 2 extractions at 250 and 300atm. Figures 3, 4 and 5 show chromatograms of extracts of the same ball pen ink at 250, 300 and 350 atmospheres respectively. In this case however, the ink has been on the paper for 7 months. In comparison to the 1 month old ink sample, the 7 month old sample shows the presence of ink components in the 350 atmosphere extract. The higher pressures are probably necessary to remove the in this case since it is more strongly bound to the paper being older than the 1 month old sample. Figures 6,7 and 8 again show 250, 300 and 350 atm extracts of the same ball pen ink, in this case 12 months old. Again for the older ink, 350 atmospheres of pressure are required to remove all of the ink from the paper.

Now let us compare the differences in the profiles of inks of different ages. In particular consider figures 2, 4 and 7 which represent 300 atm extracts of the same ink 1,7 and 12 months respectively after being applied to the paper. There are significant differences in both the number of peaks and peak height ratios.

Similar results have been obtained with the other inks of different ages in our collection, leading us to believe that supercritical fluid extraction at different pressures followed by HPLC analysis has great potential in the determination of the age of a document.

5. CONCLUSION

This research approach was based on the previous work of Dr Cantu of the Secret Service, in which he used a high polarity solvent to extract all of the ink and a weaker polarity solvent to extract a percentage of the ink. He then determined amount of selected components extracted by each solvent and measured the ratio of one to the other. His theory is that these ratios will change with time, thus allowing the age of the writing to be estimated.

Cantu had the problem of reproducibility, since each step was done manually, every procedure has to be carefully timed. We have effectively removed the problem of reproducibility of the extraction by using supercritical fluids. The instrument can be programmed to control the temperature, pressure and time of the extraction. Since manual steps have been eliminated the extraction procedure is standardized and reproducible.

Once extracted any suitable method can be employed for the analysis of the samples. We have concentrated on the use of HPLC for the examination of ink extracts since it offered the required sensitivity and specificity for our purposes but other chromatographic, spectroscopic or electrophoretic techniques may also be appropriate.

6. REFERENCES

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Waters 991 Chromatogram calculation Waters

Y-scale .121 AU/FS
Paper speed 20 mm/min
Column mm ID * mm
Packing material
Time range 0 --- 8 min
Mobile phase
Interval 1.54 sec
Flow rate ml/min
Baseline OFF
Pressure

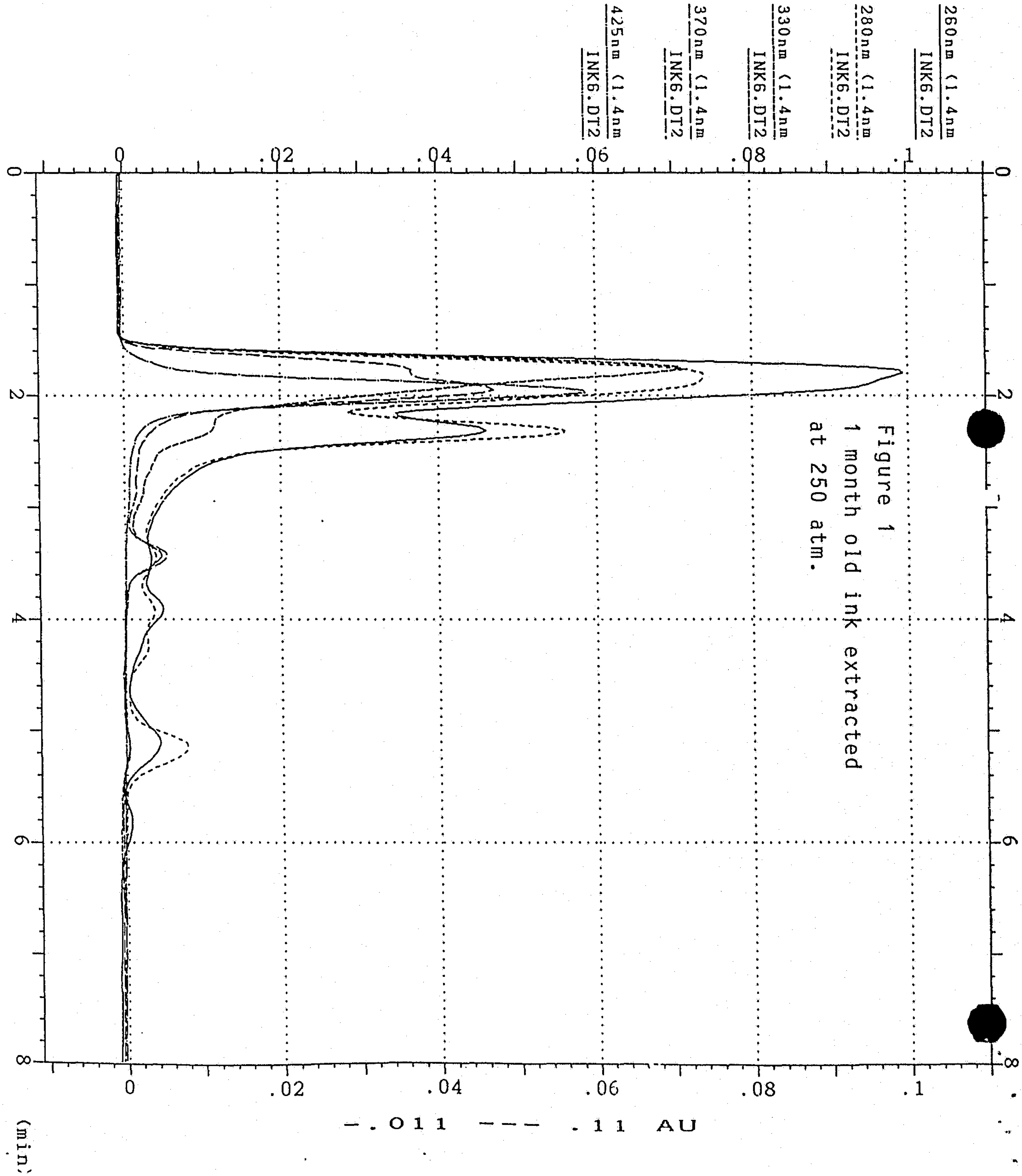

Figure 1
1 month old ink extracted
at 250 atm.

Waters 991 Chromatogram calculation Water

Y-scale .0051 AU/FS
Paper speed 20 mm/min
Column mm ID * mm
Packing material
Time range 0 --- 8 min
Mobile phase
Interval 1.54 sec
Flow rate ml/min
Baseline OFF
Pressure

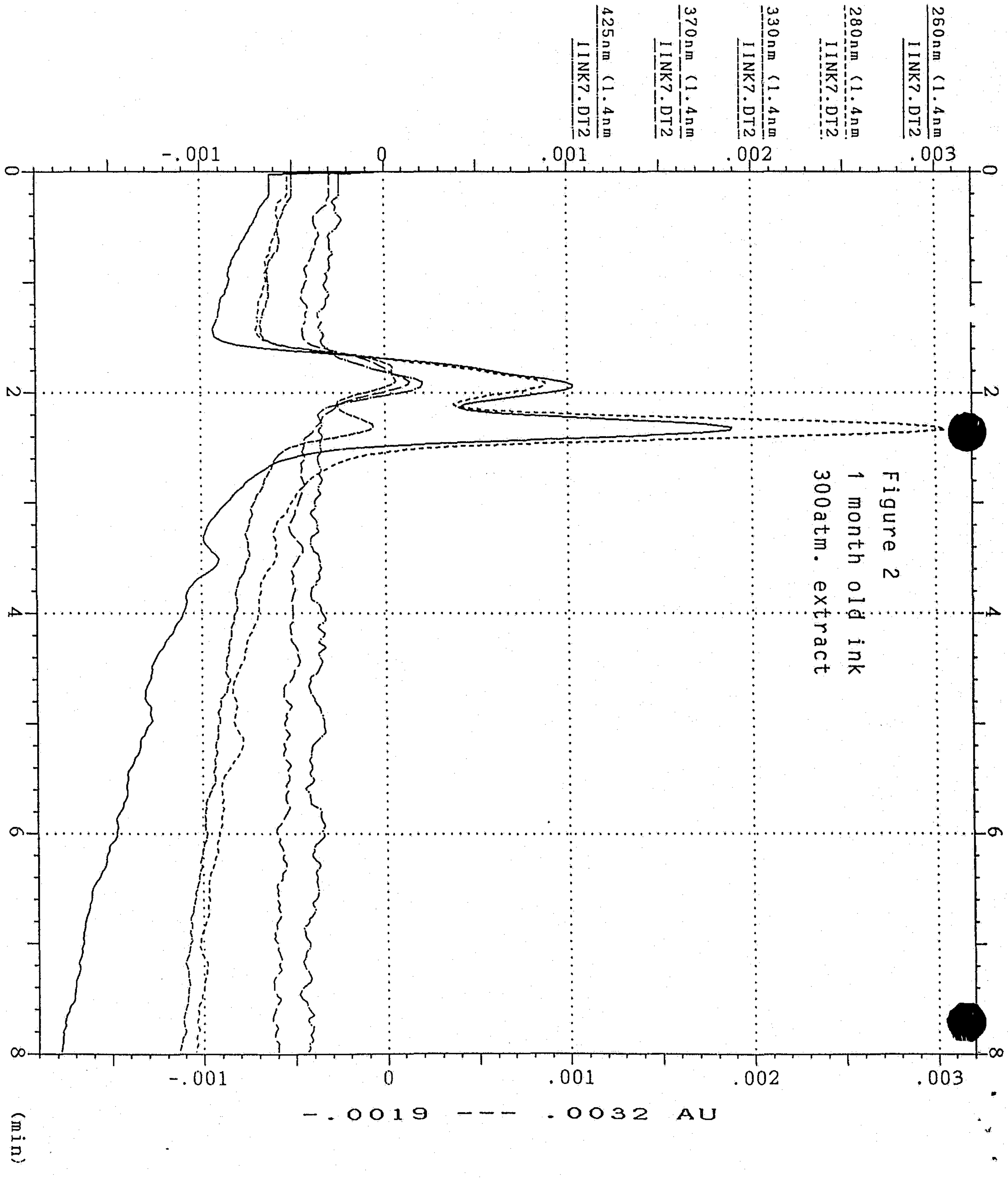

Figure 2
1 month old ink
300atm. extract

1 month 300

Waters 991 Chromatogram calculation Waters

Y-scale .028 AU/FS
Paper speed 20 mm/min
Column mm ID * mm
Packing material
Time range 0 --- 8 min
Interval 1.54 sec
Baseline OFF
Mobile phase
Flow rate ml/min
Pressure

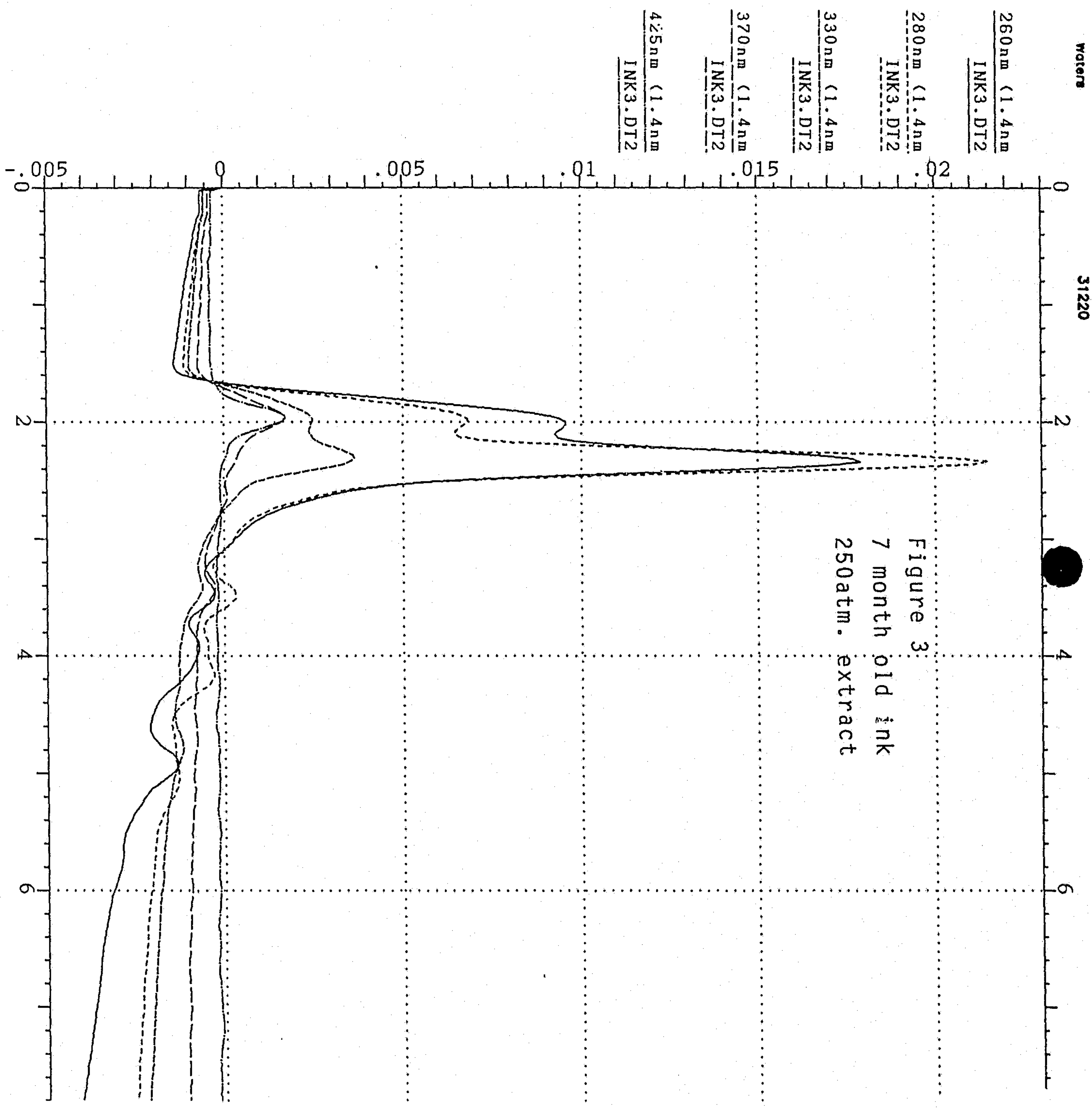

Figure 3
7 month old ink
250atm. extract

7 months

nin)

Waters 991 Chromatogram calculation Water

Y-scale	9.600001E-03 AU/FS	Paper speed	20 mm/min
		Column	mm ID * mm
		Packing material	
Time range	0 --- 8 min	Mobile phase	
Interval	1.54 sec	Flow rate	ml/min
Baseline	OFF	Pressure	

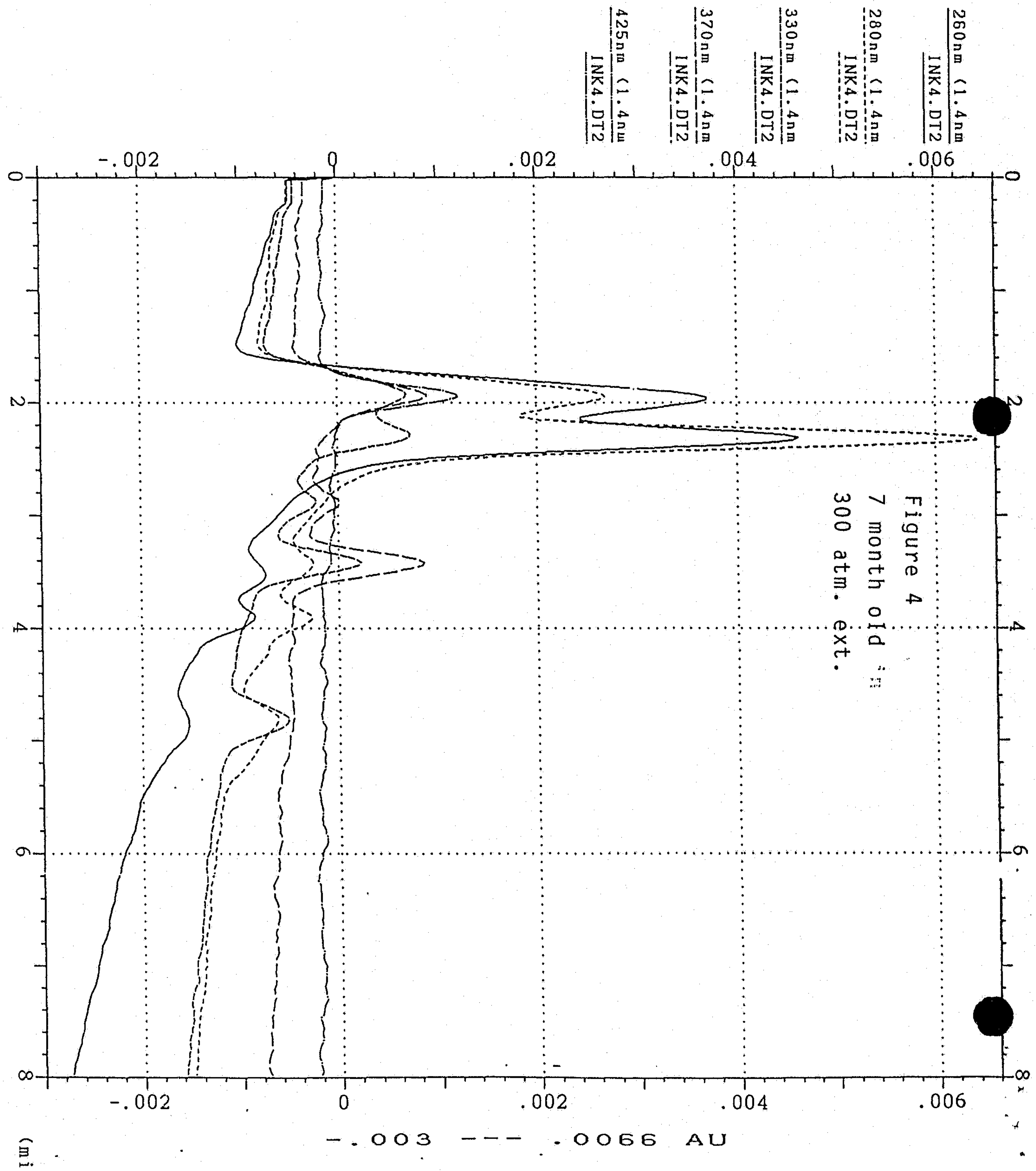

Figure 4
7 month old
300 atm. ext.

7 month
300

Waters 991 Chromatogram calculation Waters

Y-scale	.1001 AU/FS	Paper speed	20 mm/min
		Column	mm ID * mm
		Packing material	
Time range	0 --- 8 min	Mobile phase	
Interval	1.54 sec	Flow rate	ml/min
Baseline	OFF	Pressure	

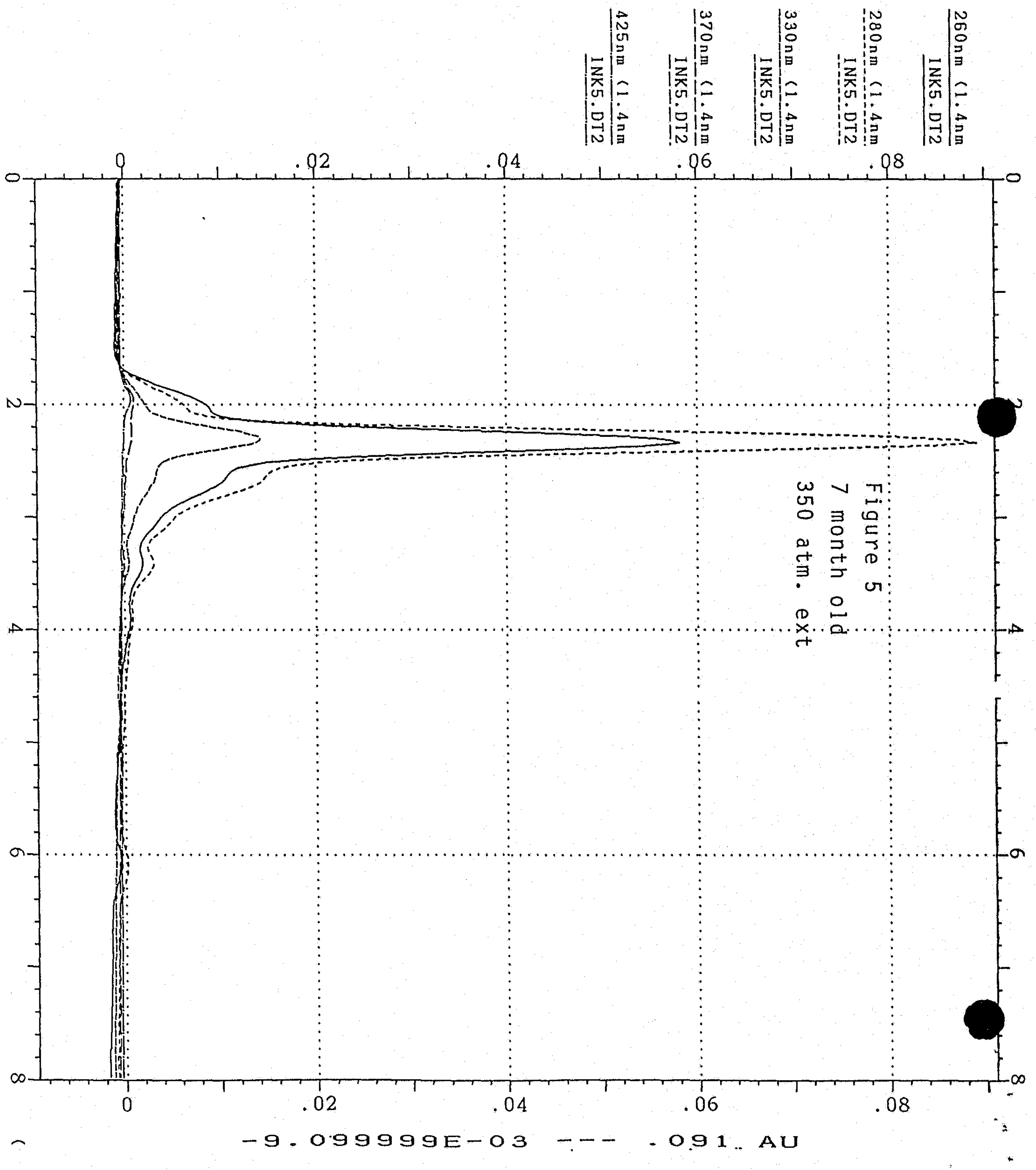

Figure 5
7 month old
350 atm. ext

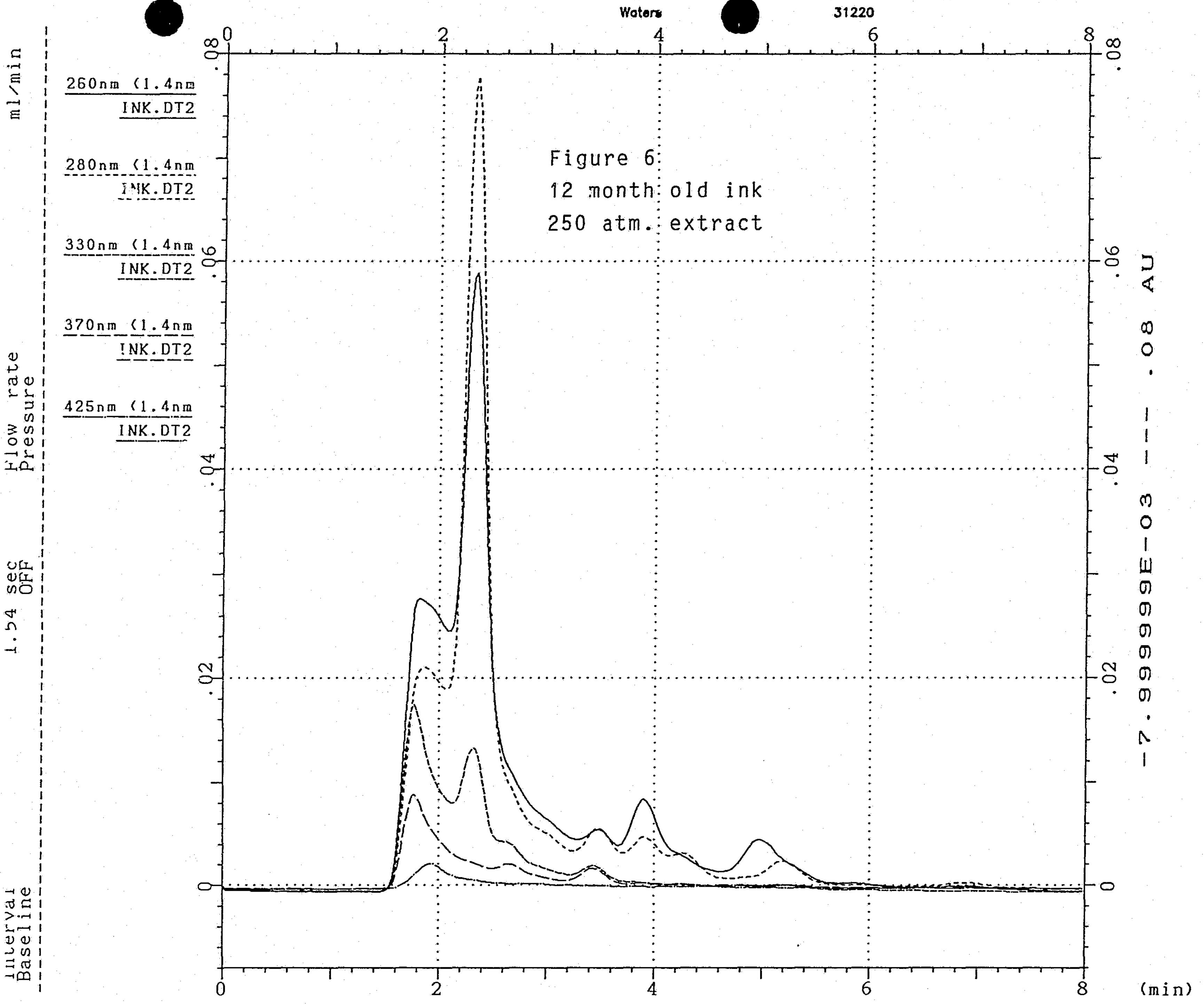

Figure 6
12 month old ink
250 atm. extract

12months

Paper speed 20 mm/min

Column mm ID * mm
Packing material

Time range 0 --- 8 min
Interval 1.54 sec
Baseline OFF

Mobile phase
Flow rate ml/min
Pressure

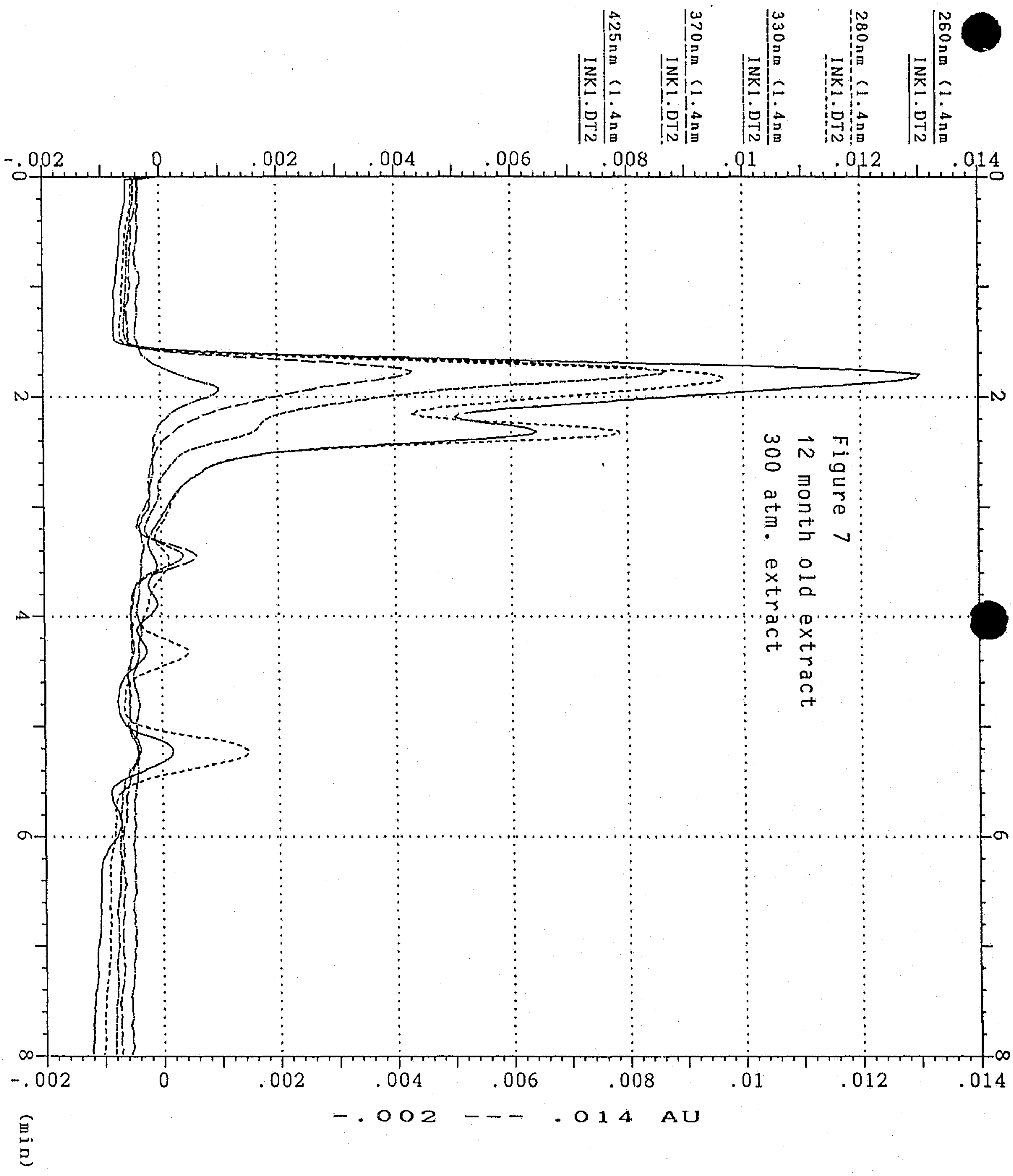

Figure 7
12 month old extract
300 atm. extract

12mths
300

Waters 991 Chromatogram calculation Waters

Y-scale .088 AU/FS Paper speed 20 mm/min

Waters 991 Chromatogram calculation Wate

Y-scale	.0015 AU/FS	Paper speed	20 mm/min
		Column	mm ID * mm
		Packing material	
Time range	0 --- 8 min	Mobile phase	
Interval	1.54 sec	Flow rate	ml/min
Baseline	OFF	Pressure	

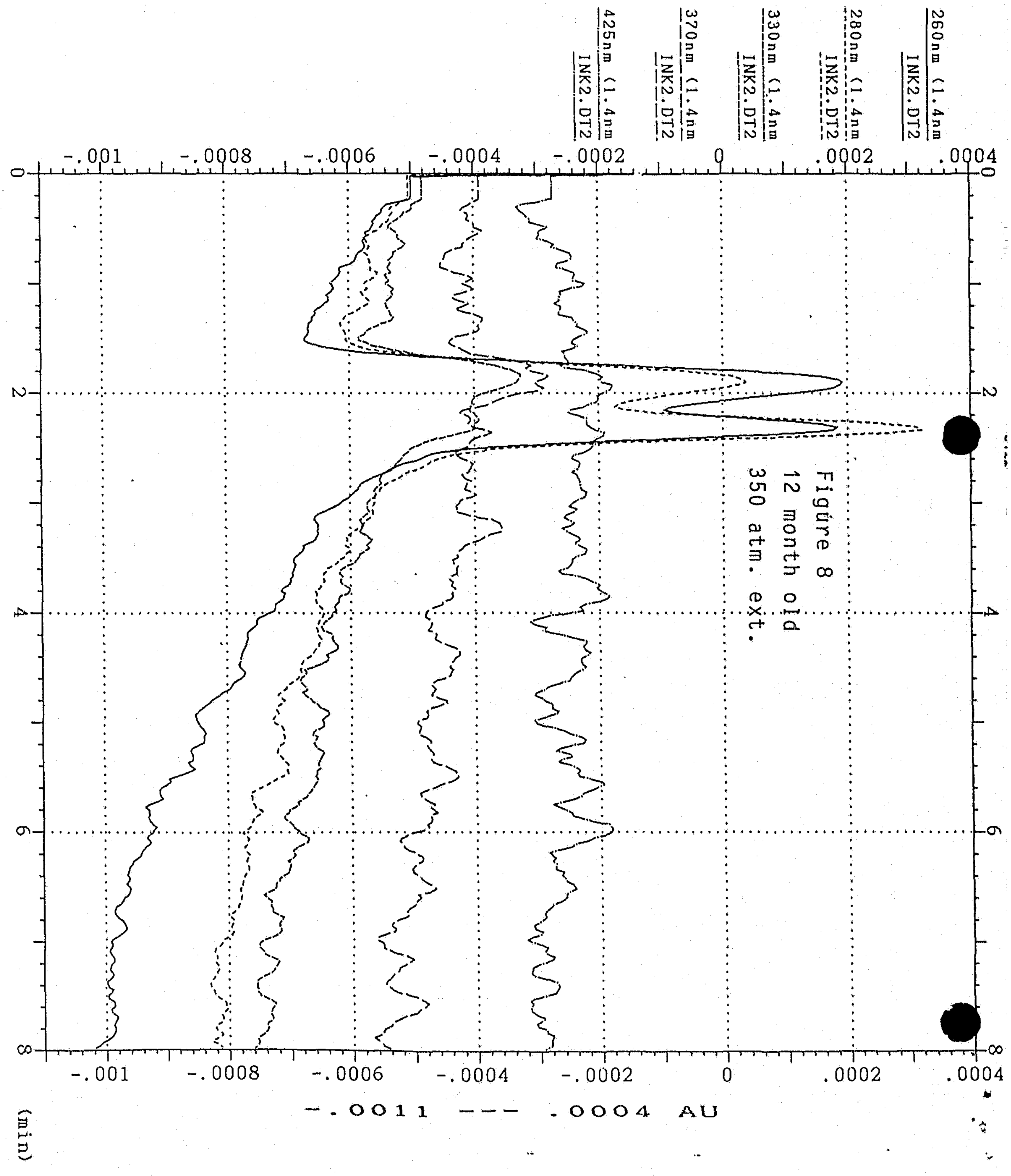

Figure 8
12 month old
350 atm. ext.

12 months
350