

Radioactive Chromatography: Determining the Smallest Detectable Peak?

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Accurately determining the limit of detection in radioactive chromatography using digital recording is useful for a range of pharmaceutical applications.

Radioactivity detection is strongly influenced by the statistical variation of the detected signal of radioactivity. Radioactivity is detected in continuous flow in liquid chromatography (LC) and gas chromatography (GC), or along a trace for thin-layer chromatography (TLC). This results in statistical variations when the interval time of the recording is too small.

Small peaks of small radioactive fractions can be hidden in the "grass" of statistical variations.

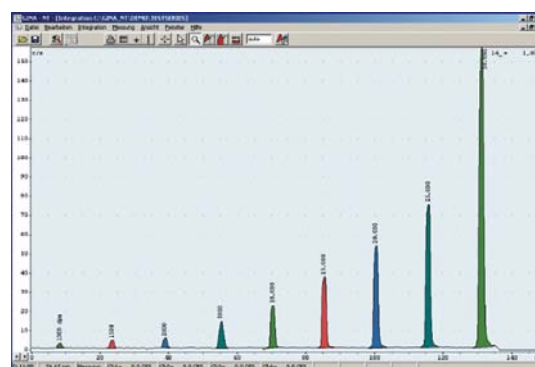
When the statistical variation is reduced by the selection of a long interval time, the baseline may be smooth, but small peaks may be "ironed out" and not discovered. Therefore, the ability to isolate the minimum detectable fraction in a radioactive chromatogram is of paramount importance.

Radioactive Chromatography

Radioactive chromatography (RC), in terms of separation performance of various fractions, is the same as conventional chromatography. The appropriate LC or GC column or TLC plate is selected for its separation performance for a particular sample mixture. Chromatographic methods such as gradient elution and injection- or spotting-technologies can also be developed to optimize the separation.

The peak width at half maximum (FWHM) for the selected column or TLC plate is known and/or can be determined from the chromatogram under evaluation. Usually the separation performance is deteriorating from the start to the solvent front

Figure 1: Test radio-chromatogram on a TLC plate.



and a mean resolution along the chromatogram (peak width) can be determined. From the separation performance expressed in FWHM of a peak the foot width of that peak can be calculated. The foot width of a peak determines the region of interest on the baseline to identify a peak in a chromatogram.

Statistic of Radioactivity Detection

Measurement of radioactivity presents a number of obtained

Figure 2: Test radio-chromatogram with 245, 196, 147, 98 and 49 dpm/injection.

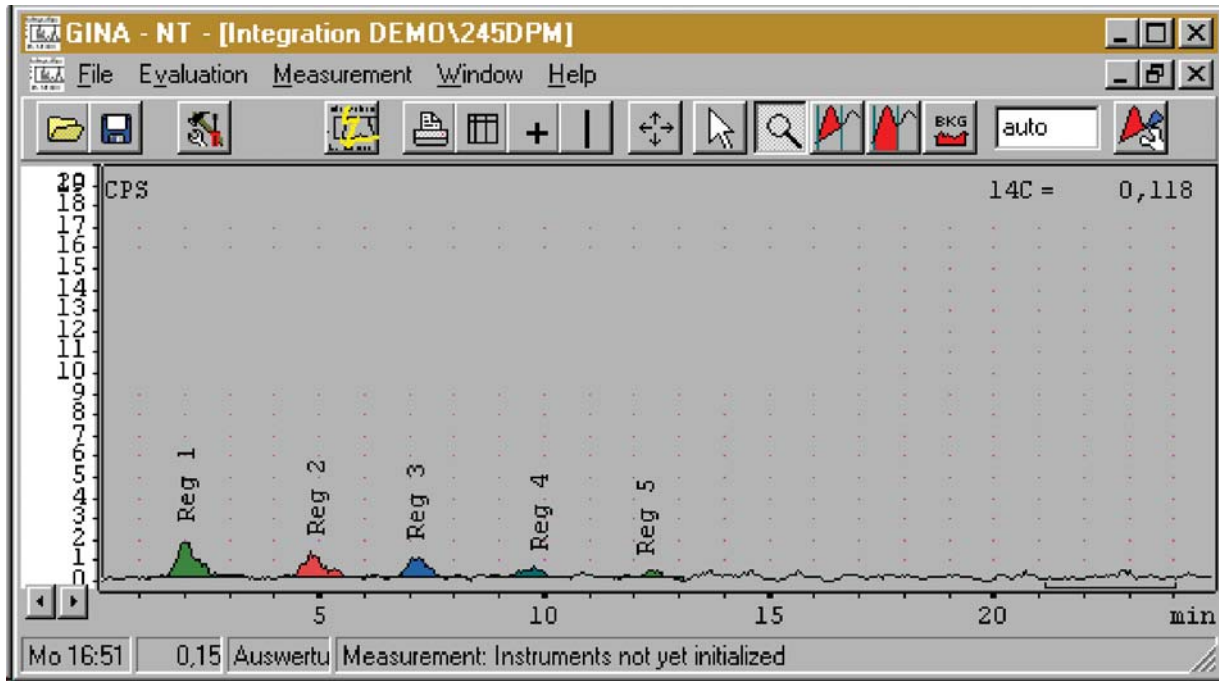


Figure 3: Test report with 245, 196, 147, 98 and 49 dpm/injection.

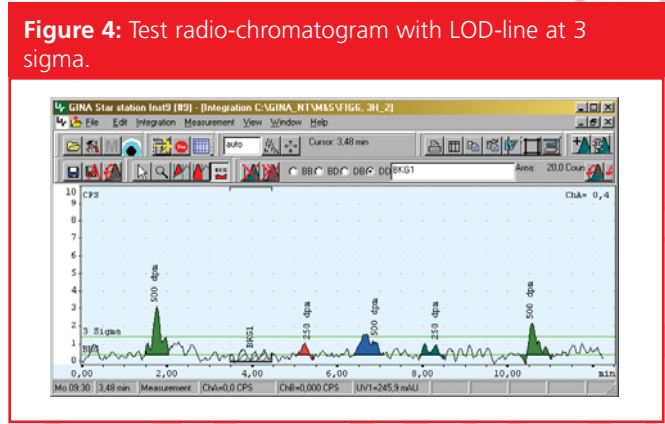
The report window shows the following data:

Substanz	Ret	Typ	Counts	C-Error
Reg 1	1,97	DD	57,9	± 9,2
Reg 2	4,88	DD	40,9	± 8,2
Reg 3	7,13	DD	27,3	± 7,1
Reg 4	9,87	DD	14,7	± 6,0
Reg 5	12,23	DD	4,3	± 5,0
Total			144,9	
Total area			162,7	
BKG1			0,20 CPS	

events during the measurement time. The number of obtained events varies according to the law of statistical fluctuation.

The range of statistical variation can be calculated from the number of obtained events.

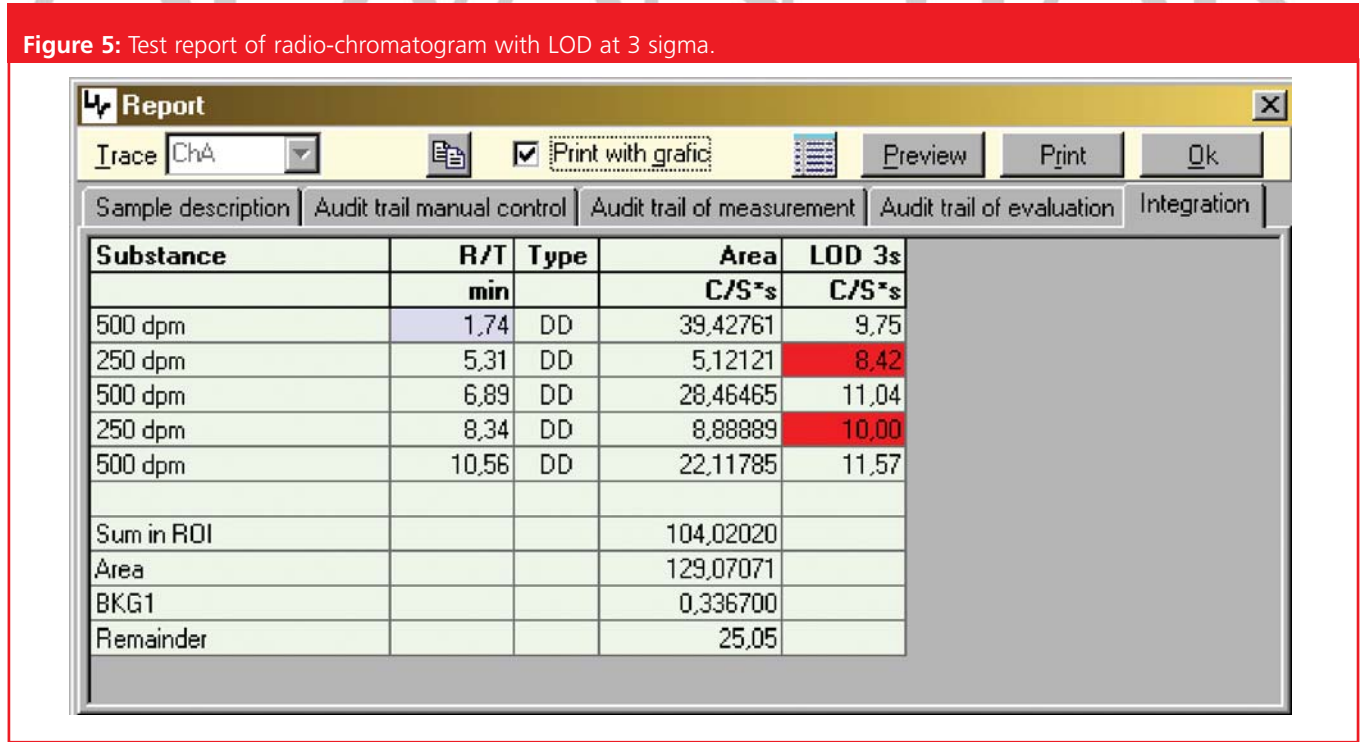
Sigma is the square root of the mean number of events. The range of statistical variation of ± 1 sigma covers 66% of all possible deviations from the mean value, ± 2 sigma covers 95% and ± 3 sigma covers 99.5% of all variations of the mean value.



Search for the Minimum Peak

During the recording of a radio chromatogram the computer is continuously calculating a sliding mean value for a region, which is 2.5 times the FWHM-value. When the FWHM value is deteriorating from start to front, the mean peak-foot-width for the total chromatogram length has to be calculated or a suitable list or function of individual peak-foot-regions along the entire chromatogram has to be created.

For every individual peak-foot-region the number of detected events of background is integrated. The user can select the number of sigma for the determination of the statistical probability to define a peak. The computer calculates the defined statistical variation along a chromatogram.



When the detected number of events is larger than the background mean value plus the n sigma value, this region has to be considered to be a small peak with the probability according to the selected number of sigma values.

Identification of a Small Peak

To identify a small peak easily, the value of the limit of detection (LOD) is calculated and printed as a decision marker line in the radio-chromatogram and as a marked digital value in the table of results for every peak. If the individual peak integral is larger than the selected statistical variation of a peak the user can easily confirm that this peak is a real peak. For easy optical support to find a small peak, a line can be calculated, drawn and printed, which symbolizes the selected sigma value above which peaks have to be considered to be a real peak and not statistics.

Digital Radio Chromatogram Recording

Conventional radioactivity detectors for chromatography are connected to conventional chromatography systems by the formation of an analogue output signal of 0–1 V. The analogue output voltage of 0–1 V for a conventional radio chromatography detector (RCD) is created by a digital rate meter. The number of events counted for a particular interval time is converted by the digital rate meter for a particular range and interval time to an analogue output of 0–1 V. The recorded chromatogram shows millivolts (mV) on the y-axis and seconds (s) on the x-axis. The peak integral is $\text{mV} \cdot \text{s}$. This peak area has to be calibrated to Becquerels (Bq) for every range and interval time of the used rate meter setting. This is very laborious and, therefore, often neglected. When the user decides on an easy analogue recording of 0–1 V, the possibility of determining LOD is permanently lost. Only digital recording and digital chromatogram storage enable the calculation of the LOD and identification of the smallest detectable peak.

Value of LOD in Radio Chromatography

Digital recording of radioactivity is a reliable way to obtain maintain, evaluate and reproduce the desired quality of results for applications that require good laboratory practice (GLP) and good manufacturing practice (GMP). In metabolism studies very small fractions must be identified and determining LOD using digital recording is immensely helpful. Digital recording is useful when synthesizing radioactive compounds as very high purity is required so minute impurities need to be determined

and identified. It is also useful in pharmacokinetic studies where tiny fractions of the compound have to be found and identified from very low concentrations of sample compound.

Günter Dietzel gained his Dipl. Ing in nuclear process technology in 1964 at the University of Essen. In 1980 he became the founder and CEO of Raytest Isotopenmessgeräte, Straubenhardt, Germany.

Reinhard Grugel is a physicist who studied at the University of Münster, Germany. Since 1981 he has been involved in the development of nuclear instruments and programmes and was promoted to senior manager of the Raytest development team in 1998.

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